Middle East Edition

Basic & Clinical Immunology

Sixth Edition

Daniel P. Stites
John D. Stobo
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1987

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Notice: Our knowledge in clinical sciences is constantly changing. As new information becomes available, changes in treatment and in the use of drugs become necessary. The authors and the publisher of this volume have taken care to make certain that the doses of drugs and schedules of treatment are correct and compatible with the standards generally accepted at the time of publication. The reader is advised to consult carefully the instruction and information material included in the package insert of each drug or therapeutic agent before administration. This advice is especially important when using new or infrequently used drugs.

The discussions in this text are not intended to serve as a manual of clinical treatment; where specific medications or drug dosages are mentioned, the physician should also consult more comprehensive medical texts.

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Preface

Five editions of *Basic & Clinical Immunology* have established it as a valuable textbook for students in the health sciences and for health professionals. Designed to be both authoritative and readable, it features essential coverage of basic principles as well as up-to-date treatment of laboratory medicine and clinical immunology. The sixth edition continues in this tradition.

Organization

The book is divided into three sections. **Section I** describes basic principles of lymphocyte biology, the structure and function of immunoglobulin molecules and their genes, the function of soluble mediators in cells capable of amplifying immunologic reactivity, and immunogenetics. **Section II** consists of three chapters on laboratory immunology that show how the principles discussed in Section I can be applied to problems of diagnosis and management. The clinical chapters in **Section III** focus on primary immunologic diseases or on disorders with important immunopathologic characteristics. This balanced presentation provides a solid foundation in the most important aspects of modern immunology.

New Features

All chapters and references have been carefully updated. In addition, the sixth edition of *Basic & Clinical Immunology* includes the following new features:

- An overview of developments in immunology from 1960 to 1985 (Chapter 2).
- A discussion of the use of monoclonal antibodies in the design of immunomodulating drugs (Chapter 16).
- A discussion of tumor immunology (Chapter 14).
- Completely revised chapters on laboratory medicine, including new discussions of binder-ligand assays, predictive value theory, and immunologic testing in Chapter 17.
- A new chapter on clinical transplantation (Chapter 23).
- Extensive revision of the chapters on hematologic, neurologic, parasitic, and pulmonary diseases, and reproductive immunology.

The authors are pleased to report that Spanish, Italian, and Portuguese editions of this text have been published, and French and Serbo-Croatian translations are in process.

We welcome comments and suggestions from our readers and appreciate any suggestions for improvements and corrections.

—The Editors

December, 1986

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Section I. Basic Immunology

The Historical Background of Immunology

1

Pierre Grabar, DSc

Immunology is a relatively young branch of medical science. Many observations of importance to immunology were made by microbiologists around the turn of this century, usually in the course of active research in bacteriology and infectious diseases. For many years immunology was studied as part of microbiology, and progress in the field consisted mainly of application of what had been learned about immunologic phenomena to the problems of the diagnosis and control of bacterial infections. Some of the most important advances were made possible by the introduction of chemical techniques in the elucidation of the nature of antigens and antibodies.

The explosive increase in fundamental information has made immunology an independent branch of science. *Zeitschrift für Immunitätsforschung* began publication in 1909 and the *Journal of Immunology* in 1916. There are now 27 national member societies in the International Union of Immunological Societies. This chapter will outline some of the contributions by pioneers in immunology that have led to the current state of the art. Where appropriate, reference is made to relevant chapters in this book.

The term **immune** derives from Latin *immunis*, ie, exempt from "charges" (taxes, expenses). However, for nearly a century the term immunity has denoted resistance to possible attack by an infectious agent. Resistance to second attacks of certain diseases had been observed even in ancient times. Attempts to protect against variola (smallpox) were made in ancient China before our era and in western Asia by inoculation (variolation) using vesicle fluid from persons with mild forms of smallpox, or by purposely seeking out contact with diseased individuals. Lady Mary Wortley Montagu (1721) introduced into England from Turkey the process of **variolation,** or inoculation with unmodified smallpox virus. It was quite dangerous, since disease and death often resulted. Similarly, an ancient Greek king of Pontus, Mithridates VI, tried to protect himself against the effects of poison by administering small amounts of poisonous substances on multiple occasions—a procedure that came to be called **mithridatism.**

A Portuguese army officer, Serpa Pinto, who traveled through central Africa in the middle of the last century, related how local "wizards" protected people against snakebites by treatment with a mixture of snake heads and ant eggs. At the beginning of this century, the same procedure was employed by specialists called "djoekas" among the black population of Dutch Guiana. It is interesting that ants contain formol, which is now used for the detoxification of toxins and venoms.

EARLY IMMUNOLOGY

The first effective—though still empiric—immunization was performed by Edward Jenner, an English physician (1749–1823), who observed that persons who got well after infection with cowpox were protected against smallpox. Jenner introduced vaccination with cowpox in 1796 as a means of protecting against smallpox. The term **vaccination** (L *vacca* cow) was introduced to replace the term variolation.

The scientific approach was not applied to the study of immunologic phenomena until almost a century later as a consequence of work on microbes by Louis Pasteur (1822–1895) and his collaborators. They investigated the possibility of protecting against infection by vaccinations with attenuated strains of microorganisms. Their first observation (1878–1880) was that a culture of *Pasteurella aviseptica* (then called chicken cholera) which had been left in the laboratory during vacation lost its virulence for chickens and that animals inoculated with this culture were protected against the virulent strain. Pasteur concluded that this culture contained attenuated microbes and, to honor the work of Jenner (nearly 100 years before), extended the term vaccination to denote conferring immunity by injection of attenuated strains of organisms. The idea of using attenuated strains of microorganisms was confirmed by Pasteur when he studied vaccination against anthrax (1881). Research on the mechanisms of protective effects led Richet and Hericourt to the observation (1888) that the blood of an animal immunized with staphylococci conferred partial protection against subsequent inoculation with these microorganisms. The next year, Charrin and Roger observed that the serum of an animal immunized with *Pseudomonas aeruginosa* (then called *Bacterium aeruginosum* among other names) agglutinated a suspension of this microbe.

In 1889, Pfeiffer, a pupil of Koch, used cross-im-

munization of guinea pigs with 2 similar microbes (*Vibrio cholerae* and *Vibrio metchnikovii*) to show that it was possible to distinguish them immunologically, since immunization against one did not protect against the other. The specificity of the protective effects of immunization had already been observed, but this example showed how extremely fine the specificity could be in some cases.

"CELLULAR IMMUNITY" THEORY

In 1882 in Messina, the Russian zoologist Elie Metchnikoff (1845–1916) studied the role of motile cells of a transparent starfish larva in protection against foreign intruders. He introduced a rose thorn into these larvae and noted that a few hours later the thorn was surrounded by motile cells. This experiment can be considered the starting point of cellular immunology. It had already been established by Koch and Neisser that bacteria can be found in leukocytes, but it was thought that this was the result of bacterial invasion of the leukocytes. Metchnikoff showed that the leukocytes had in fact engulfed the microorganisms. In 1883, Metchnikoff observed that *Daphnia*, a tiny transparent metazoan animal, can be killed by spores of the fungus *Monospora bicuspidata* and that in some instances these spores are attacked by blood cells and can be destroyed in these cells, thereby protecting the animal against the invaders. In 1884, he extended these observations to the leukocytes of rabbits and humans, using various bacteria. He noted that the engulfment of microorganisms by leukocytes, which he called **phagocytosis,** is greatly enhanced in animals recovering from an infection or after vaccination with a preparation of these microorganisms. He therefore concluded that phagocytosis was the main defense mechanism of an organism. He later showed the existence of 2 types of circulating cells capable of phagocytosis—the polymorphonuclear leukocytes and the macrophages—as well as certain fixed cells capable of phagocytosis, and proposed the general term **phagocytes** for all of these cells (Chapter 9).

The **cellular immunity** theory of Metchnikoff, who worked at the Pasteur Institute in Paris from 1887, was accepted with enthusiasm by some but was criticized by several other pathologists. The inflammatory reaction had been described by Celsus as early as the first century AD, but before Metchnikoff it had been studied only in mammals. Pathologists such as Virchow (1871) agreed that inflammation was due to changes in the connective tissue cells induced by various agents, particularly by abnormal deposits of metabolic products. Cohnheim (1873) and his collaborator Arnold (1875) considered inflammation to be a local vascular lesion due to a noxious agent which allowed blood cells to penetrate into tissues. Metchnikoff, who had observed the same accumulation of motile cells in lower animals with no circulatory vessels, asserted that diapedesis in higher animals was a process of active penetration of these cells through the walls of the vessels (1892). In his opinion, inflammation resulted from an enzymatic digestion process due to ingestion of the noxious agent by the motile phagocytes.

"HUMORAL" THEORY

Metchnikoff's theory came under severe criticism somewhat later by those who observed immunity in the absence of cells. Fodor in 1886 was apparently the first to observe a direct action of an immune serum on microbes during the course of his studies on anthrax bacilli. Behring* and Kitasato (1890) demonstrated the neutralizing antitoxic activity of sera from animals immunized with diphtheria or tetanus toxin, which was considered the first proof of humoral immunity. In 1894, Calmette observed the same neutralizing activity of snake venom antiserum.

An important humoral defense mechanism described by Pfeiffer and Isaeff (1894) has come to be called the **Pfeiffer phenomenon.** Cholera vibrios injected into the peritoneum of previously immunized guinea pigs lose mobility, are clumped, are no longer stainable, and are later phagocytized by leukocytes, but they are also lysed in the absence of cells.

A theory of immunity due to humoral factors provoked intense debate between Metchnikoff and the supporters of this new theory, mainly from the laboratory of Robert Koch (1843–1910). At the time of Pfeiffer's discovery, a young Belgian, Jules Bordet (1870–1961), was engaged in the study of agglutination reactions in Metchnikoff's laboratory at the Pasteur Institute. He became interested in the Pfeiffer phenomenon and in 1895 showed that both bacteriolysis and lysis of red cells (which he described in 1898) required 2 factors: one, which he called **sensitizer,** was thermostable and specific; the other, which he called **alexin,** was thermolabile and nonspecific. The factor designated alexin by Bordet came to be called **cytase** by Metchnikoff and **complement** by Ehrlich (see Chapter 10). Bordet believed that his "alexin" possessed enzymatic activity and that it consisted of several components.

It is of interest that Bordet's studies of humoral factors were performed in Metchnikoff's laboratory and were in contradiction to the master's theories. Later, both theories gained general acceptance and it was established that humoral factors originated from lymphoid cells.

During this period, the term **antigen** was introduced to designate any substance (then mainly microbes or cells) capable of inducing a reaction against itself and the illogical term **antibody** (both being "anti-") to designate the factor present in the serum possessing this activity. At first, various special names were used to indicate each observed antibody activity,

*The particle von was added later to Behring's name after he became famous—about the time he received the Nobel Prize.

such as **agglutinins, precipitins, sensitizers,** and **opsonins.** The first observation of agglutination is described above. The precipitin reaction was described later—in 1897 by Kraus with microbial culture supernates and the serum of immunized animals, and in 1899 by Tchistovitch with serum protein antigens and by Bordet with milk antigens and serum of animals injected with these fluids. The precipitin reaction was introduced by Wassermann and Uhlenhuth into forensic medicine for the identification of blood or meat.

Resolution of Conflicting Theories

In 1895, Denys and Leclef observed the fixation of antibodies present in an antistreptococcus serum by these organisms and called them **bacteriotropins.** Neufeld and Rimpau had also demonstrated similar in vitro fixation. In 1903, Wright and Douglas, after a careful study of Metchnikoff's observation that phagocytosis of microbes is facilitated by the serum of an immunized animal, used washed cells to demonstrate that the immune serum contained an active factor they called **opsonin.** They proposed the term **opsonization** for the activity, and this phenomenon acted as a "bridge" between the apparently contradictory humoral and cellular theories.

During this same period, Paul Ehrlich (1854–1915) studied the neutralization of toxins by immune serum, using the highly toxic vegetable poisons abrin and ricin, which could be extracted easily in sufficient quantity. These studies enabled him to establish a technique for the evaluation of the antitoxic activity of diphtheria antiserum (1897). In 1908, Magnus induced immunologic techniques in plant taxonomy.

EHRLICH'S "SIDE-CHAIN" THEORY

Ehrlich was interested in the theoretic aspects of immunologic phenomena and in 1896 elaborated his **side-chain theory** to explain the appearance of antibodies in the circulation. He considered it an "enhancement" of a normal mechanism and suggested that cells capable of forming antibodies possessed on their surface membranes specific side chains which were receptors for antigens. He proposed that binding of antigen to the side chains provoked new synthesis of these side chains, which were liberated into serum as antibodies. He expressed the specificity of the reaction of antigens and antibodies as a "key antigen in a lock antibody" and thought that this reaction was of a chemical nature. During the next few years, he tried to substantiate his theory with various arguments, but the theory was not generally accepted. It was criticized by Bordet, who felt that the antigen-antibody reaction was of a colloid nature; by Gruber; and particularly by Arrhenius and Madsen, who insisted on the reversibility of the reaction and on different proportions of reactants in specific precipitates. Nevertheless, Ehrlich's general theory, with modifications and additions, has been taken into consideration by many authors, and his hypothesis on the existence of specific receptors on immunocompetent cells has recently been completely vindicated.

Isoantibody

In 1875, Landois published his monograph Blood Transfusion. He noted the effects of blood transfusions between members of different species and observed it was preferable to work within a single species. He also stated, however, that there were differences within a single species, since a recipient's own cells could be hemolyzed by serum from a nonidentical donor of the same species.

The term **isoantibody** or **isohemagglutinin** was introduced by Bordet, who observed in 1898 that the serum of rabbits injected with red cells of another species agglutinated the red cells whereas rabbit red cells injected into rabbits were not agglutinated. However, in 1902, Landsteiner used the agglutination reaction to demonstrate several different antigenic specificities of red cells in the same species—the blood groups A, B, and O in humans—which became the basis of blood transfusion (Chapter 22). Later, he also discovered Rh specificity, using rhesus monkey blood. The term isoantibody is no longer used for antibodies to antigenic determinants specific for other species. It is now used to indicate antibody in an individual to antigenic determinants in other genetically nonidentical members of the same species, eg, anti-A antibody (isohemagglutinin) in blood group B humans (see Chapter 22).

Ehrlich also observed that the plant toxins abrin and ricin agglutinate red cells. Landsteiner and Raubitchek in 1907 extended these observations, using particularly Papilionaceae (a family of beans). These plant-derived hemagglutinins were later termed **lectins** by Boyd.

Hypersensitivity

At the close of the 19th century, all of the immunologic phenomena observed to that time supported the view that they were defense mechanisms. Apparent contradictions were the observations of Landsteiner and, particularly, the discovery of anaphylaxis by Richet and Portier in 1902. It had already been shown, particularly by Wassermann and von Dugern, that second challenge of a previously immunized organism with the same antigen increased the antibody activity in its serum. Thus, the fact of immunologic memory had to be explained. The discovery of Richet and Portier was absolutely unexpected. They studied the toxic activity of the tentacles of Actinaria by injecting a glycerin extract into dogs. The first injection, in small doses, had no direct observable effect, and they thought the animals were protected. But a second injection resulted in shock—often lethal for the animals. They proposed the term **anaphylaxis** for this phenomenon (Chapters 15 and 24). The next year, Arthus described what is now called the **Arthus phenomenon,** ie, the local necrotic lesion produced by injecting antigen into a previously immunized animal. This reaction is specific, whereas an analogous but

AN

INQUIRY

INTO

THE CAUSES AND EFFECTS

OF

THE VARIOLÆ VACCINÆ,

A DISEASE

DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,

PARTICULARLY

GLOUCESTERSHIRE,

AND KNOWN BY THE NAME OF

THE COW POX.

BY EDWARD JENNER, M.D. F.R.S. &c.

——QUID MORIS CERTIUS ESSET
ADPULSUS RERE POTENT, QUO VERA AC FALSA NOTEMUS.
LUCRETIUS.

London:
PRINTED, FOR THE AUTHOR,
BY SAMPSON LOW, N° 7, BERWICK STREET, SOHO
AND SOLD BY LAW, AVE-MARIA LANE; AND MURRAY AND HIGHLEY, FLEET STREET
1798.

Figure 1–1. Face plate from first edition (1798) of Jenner's Inquiry Into the Causes and Effects of . . the Cow Pox.

Figure 1–2. Louis Pasteur (1822–1895). (Courtesy of the Museum of the Pasteur Institute, Paris.)

Figure 1–3. Robert Koch (1843–1910). (Courtesy of the Museum of the Pasteur Institute, Paris.)

Figure 1–4. Elie Metchnikoff (1845–1916). (Courtesy of the Rare Book Library, the University of Texas Medical Branch, Galveston.)

Figure 1–5. Paul Ehrlich (1854–1915). (Courtesy of the Museum of the Pasteur Institute, Paris.)

Figure 1–6. Emil von Behring (1854–1917). (Courtesy of the Museum of the Pasteur Institute, Paris.)

Figure 1–7. Karl Landsteiner (1868–1943). (Courtesy of the Museum of the Pasteur Institute, Paris.)

Figure 1–8. Jules Bordet (1870–1961). (Courtesy of the Museum of the Pasteur Institute, Paris.)

nonspecific reaction was described by Sanarelli and by Shwartzman many years later—the **Shwartzman phenomenon.**

At the beginning of the 20th century, von Pirquet, working in Vienna, studied **serum sickness,** the delayed reaction that occurred following a second injection of a heterologous antistreptococcus serum, and observed that this **hypersensitivity reaction** (von Pirquet and Schick, 1905) sometimes appeared rapidly. He suggested that this reaction had a direct connection with the presence in the animal of antibodies to the injected serum. In the course of his research on tuberculosis, he observed that a cutaneous reaction appeared more rapidly after a second injection than after the first. He developed the scratch test for tuberculin sensitivity, and in 1906 he proposed the term **allergy** for modified immune reactivity. Since then, this term has been generalized to denote all sensitization phenomena, whereas the better and earlier term **generalized anaphylaxis** is used to denote **anaphylactic shock.**

Another series of investigations on anaphylactic reactions was initiated by Smith and Otto (1906) and, more successfully, by Rosenau and Anderson in Washington (1909). These investigations showed (1) that the secondary reaction provoked in guinea pigs by the injection of diphtheria toxin and antiserum (this mixture was used at that time for vaccination) was due not to the toxin but rather to antibodies against the antiserum; (2) that the sensitizing time was about 10 days; and (3) that passive sensitization with the serum of a sensitized animal was sufficient to provoke a secondary reaction to the antigen. It was thought that the relatively long time required for sensitization to develop was due to fixation of antibodies to cells. Schultz had demonstrated in 1910 that a contractile reaction occurs in vitro following contact of the antigen with a strip of intestine of a previously sensitized animal. This reaction was also studied by Dale with uterine smooth muscle and is now called the **Schultz-Dale reaction** (Chapter 15).

Hay fever was a recognized disease entity for a long time, but until the beginning of this century it was believed to be due to toxic substances in pollen. Experimental "desensitization" was attempted by inoculation of small amounts of pollen to neutralize the supposed toxin (Besredka, 1907; Noon and Freeman, 1911). Shortly thereafter, Wolff-Eisner suggested that hay fever might be a hypersensitivity reaction, a concept proved correct in 1921 by Prausnitz and Küstner with different antigens. The term Prausnitz-Küstner (PK) reaction is therefore used to denote the test for passive transfer of reactivity to an allergen (Chapter 15). A similar phenomenon in experimental animals, the **passive cutaneous anaphylaxis (PCA) reaction,** which allows a semiquantitative estimation of antibodies, was described much later—in 1949—by Biozzi, Mene, and Ovary (Chapter 15).

The role of histamine and related substances in inflammatory and anaphylactic reactions is discussed in Chapters 15 and 24, but it is appropriate to cite a few of the more important contributions. Dale and Laidlaw in 1910 showed the similarities between the reactions provoked by histamine and those associated with anaphylaxis. Lewis (1927) explained the "triple response" in skin reactions, and Riley and West (1953) discovered that histamine is present in mast cells and is released by the breakdown of these cells. These observations opened a new field of research into inflammatory and anaphylactic reactions.

ANTITISSUE IMMUNE SERA

Early efforts in the field of transplantation immunology included the production of immune sera against tissue components (Lindemann) and the discovery of tissue and species specificity of antigens. In 1902, Metchnikoff and Besredka prepared antileukocyte antisera and observed that such antisera possessed cytotoxic activity against leukocytes. They also noted that injection of small amounts of antisera induced proliferation of these cells in the injected animal. Metchnikoff envisaged the use of such antisera to enhance the resistance of the organism against infections. Bogomoletz prepared antisera against all lymphoid tissues. The cytotoxic effect of such antisera has been the starting point for the recent use of "antilymphocyte antisera" for inhibition of graft rejection (Woodruff, Starzl). In either case, variable results are obtained because of the multiplicity of antigens on the injected cells and the consequent variety of antibody specificities in the resultant antisera.

The first 3 decades—until 1910—of active development of immunology as a separate branch of medical science witnessed the discovery and description of most of the fundamental immunologic phenomena, although the mechanisms underlying those phenomena were not elucidated. Although Ehrlich postulated that the immune phenomena must represent an "enhancement" of normal mechanisms, they were considered by most immunologists of the time to be part of the organism's "defense apparatus." This opinion gained force from the general assumption that the organism will react only against foreign ("not self") constituents, and Ehrlich's phrase *horror autotoxicus* emphasized his view that the organism would not react against "self" components, though he admitted the possibility of an autoreaction when the "normal regulatory mechanisms" were disturbed (Chapter 11). Actually, at that time, Metalnikoff, in Metchnikoff's laboratory, had demonstrated autosensitization in guinea pigs to their own spermatozoa, and we know now that autoantibodies exist in small amounts even in "normal" sera (see below).

Development of Vaccines

The next 3 decades—until 1940—were concerned mainly with applications and development of knowledge about immunologic phenomena, particularly in the preparation of immune sera, diagnostic reagents for clinical study of infectious disease, and vaccination programs. A few examples are Haffkine's experi-

ments with cholera vaccination in India in 1892, using himself and his collaborators as control subjects; the use of an attenuated strain of *Mycobacterium tuberculosis*, BCG (bacille Calmette-Guérin, 1908–1921); and vaccination against bacterial toxins using detoxified preparations. Several workers tried to develop a nontoxic but still immunogenic preparation by treating bacterial toxins with various chemicals. Formol was used by Eisler and Löwenstein (1915) for tetanus toxin and by Glenny (1921) for diphtheria toxin, but their preparations were not completely detoxified. Ramon in 1924 developed a method called optimal flocculation for the quantitative measurement of toxins and antitoxins which resulted in a satisfactory method of detoxification. He obtained preparations which he called **anatoxins,** now generally called **toxoids,** as proposed by Ehrlich years before.

In 1916, LeMoignic and Pinoy introduced lipid (as adjuvant) vaccines, and in 1935 Ramon obtained some good results with various other adjuvants to increase the production of antitoxins in horses, although these produced lesions at the site of the injection. These were precursors of the current main adjuvant, Freund's complete adjuvant (1947), used to augment immune responses.

The first completely synthetic antigen (diphtheria vaccine) was synthesized in 1980 by Alouf, Boquet, and Chedid.

IMMUNOCHEMISTRY

Important progress was made during the second period of immunologic studies when the principles of chemistry were applied to immunologic research. Although Ehrlich had suggested years earlier that immunologic reactions must have a chemical basis and although Arrhenius, studying antigen-antibody reactions, introduced the term immunochemistry in 1904, the applications of chemical theory and methodology truly began only during this second period.

Among the most productive applications of chemistry to immunology were the studies of Landsteiner and his collaborators (Prasek, Lampl, van der Scheer, Chase). Space does not permit discussion of their many achievements, and only one will be mentioned. In 1903, Obermayer and Pick suggested that antigens possessed the properties of immunogenicity and a capacity to react with antibodies. Subsequently, Landsteiner and his coworkers, as well as others, observed that these properties could be altered by chemical treatment of antigens (Chapter 3). This initiated in 1914 Landsteiner's studies with artificial conjugated antigens. Various chemical groupings were attached to proteins, and the specificity of these groupings was demonstrated in serologic reactions. In 1921, Landsteiner coined the term **haptens** for those specific groupings which by themselves were incapable of provoking the formation of antibodies but were still responsible for specific reaction with antibodies (Chapter 3). Similar studies were later performed by Haurowitz and Breinl (1931), who introduced groupings containing arsonate, which facilitated their recognition. Landsteiner's book *The Specificity of Serological Reactions*, published in German in 1933 and in English in 1936, had a great influence on further research, as did Wells's book *The Chemical Aspects of Immunity* (1925) and Marrack's text *The Chemistry of Antigens and Antibodies* (1935).

Immunologic Tolerance

An important observation made by Felton (1942) showed that if mice are injected with very small amounts of pneumococcal polysaccharide they are protected against infection by the corresponding microbe, but if the injection is made with large quantities of polysaccharide the mice can be infected. This **Felton phenomenon** was also called immunologic unresponsiveness and is now known as **immunologic tolerance.** The multiple mechanisms involved in this phenomenon are discussed in Chapter 11.

Identification of Immunoglobulins

Felton was probably also the first to obtain purified preparations of antibodies, using horse antisera to pneumococci and precipitating the euglobulin fraction rich in antibodies. The practical isolation of pure antibodies from such sera was achieved by Heidelberger and Kendall (1936) by dissociation of specific precipitates with concentrated salt reagents. As a result of studies by Heidelberger and Pedersen with Svedberg's ultracentrifuge (1937) and by Tiselius and Kabat with electrophoresis in liquid media (1938), it became clear that antibodies belong to that globulin fraction of the serum proteins possessing slow mobility, at that time designated γ-globulins (Chapter 4).

In parallel with the development of immunochemistry, studies on the cellular aspects of immunology had been performed mainly by hematologists and pathologists who confirmed the role of white blood cells in the formation of antibodies. Pfeiffer and Marx found that antibodies, which they called sensitizers or fixators, appear earlier in the spleen, lymph nodes, and bone marrow than in the blood. The lymphatic system, which came to be called the reticuloendothelial system, was progressively studied and various cells were described.

This period of development of the field of immunology also witnessed the isolation of the components of complement, studies on their respective activities, and identification of several specific subgroups among human and animal red cells.

RECENT PERIOD OF IMMUNOLOGY

The period of development of the discipline of immunology beginning just before World War II is characterized by the emergence of an enormous amount of new data. Space limitations preclude even brief mention of much of this work. Moreover, it is not the aim of this chapter to show the recent development and

current status of our knowledge. Therefore, only a few examples of some recent fundamental findings are briefly mentioned here.

Owen observed in 1945 that bovine dizygotic twins possess double serologic specificities. Medawar and his collaborators in London (1935) performed careful experiments on mammals, and Hasek performed similar experiments in Prague using coupled eggs of different species. Their studies on transplantation formed the basis for subsequent research on acquired immunologic tolerance and are of fundamental importance for the problem of tissue grafting (Chapter 11).

In 1948, Astrid Fagraeus showed that it is through the development of plasma cells (described in 1890 by Cajal and more recently by Gormsen and Bjorneboe) that the actual synthesis of antibodies takes place (Chapter 4). In 1953, Grabar and Williams demonstrated that immunoglobulins are heterogeneous and detected the existence of IgA (first called globulin X and then β_{2A}). The nomenclature of the immunoglobulins was established, and the amino acid sequences of many of them were studied.

Rapid progress was due particularly to the work of Porter, Edelman, Hilschman, Putnam, and others. The existence of allotypes and idiotypes of immunoglobulins was established by serologic methods (Grubb, Oudin, Ropartz, Kunkel), and the relationships between the structure of these proteins and genetic information are now being studied in many laboratories (Chapters 4 and 5).

The central role of the thymus in immunologic processes was first clearly established by experimental studies performed by Miller in London in 1961–1962 and by Waksman and Yankowic in Boston. These studies were performed with neonatal thymectomy in mice. At that time other groups were studying the role of the thymus, including Good and his group in Minnesota and Warner in Melbourne. Their initial observations and studies opened up the whole field of the cellular basis for cooperation between cells responsible for cellular and for humoral immunity, thymus-derived T lymphocytes and bone marrow-derived B lymphocytes.

The last few decades have seen the emergence of new branches of immunology:

(1) **Immunopathology** has made important contributions to our fund of knowledge and has even offered some therapeutic approaches. Studies of pathologic processes have in many ways helped us to understand normal ones, eg, absence of plasma cells in hypogammaglobulinemic children (see Chapter 20).

(2) **Immunogenetics** has included analysis of amino acid sequences in immunoglobulins (Chapter 4), histocompatibility antigens (Chapter 6), genetic markers on immunoglobulins (Chapter 4), and the absence of response to certain antigens in certain strains of animals (Benacerraf, Sela, et al). This contributes to our understanding of the transmission of genetic information and the position on chromosomes of loci controlling histocompatibility antigens.

(3) **Tumor immunology** and the immunochemical analysis of components of various human and animal tumors and leukemic cells have already clarified several important features (Chapter 14). These include the absence of various normal components on tumor cells; the appearance of antigens present normally in fetal life (Abelev; Tatarinov; Gold, Burtin, et al) or in tissues other than the one in which the tumor has developed; and the existence in some tumors of "neoantigens." The latter would imply that new genetic information has been acquired by the cells and might depend on introduction of part of a viral genome into the cell. The intensive studies will result, it is hoped, in effective forms of immunotherapy of cancers, including leukemia (Chapters 14 and 22).

(4) **Transplantation immunology** emerged from work on acquired tolerance (mentioned above). Since rejection of grafts is an immunologic phenomenon dependent mainly on the thymus, chemical substances and "antithymocyte" immune sera are being used as immunosuppressive agents. Important information has been obtained in humans and in mice from studies on histocompatibility antigens. The presence of these antigens on leukocytes makes possible histocompatibility typing (Dausset, Snell, Rapaport, 1958). International centers for human histocompatibility typing have been created to establish compatibility between tissues to be grafted (usually kidney) and the recipient. More recently, provocative associations have emerged between certain HLA phenotypes and susceptibility or resistance to disease, particularly immunologic disease.

(5) **Immunologic disorders:** The study of immune disorders is emerging as a separate discipline concerned with both "broad-spectrum" and "antigen-selective" immunodeficiency and with methods of immunotherapy for these disorders, eg, transfer factor, both broad-spectrum and antigen-selective.

(6) **Immunochemistry:** Since the work of Liener (1953) and Grabar and collaborators (1955), immunochemical methods have been used in plant physiology, in pathology, and in the agricultural industry.

Development of Techniques & Instruments

The development of new scientific information is historically related to methodologic advances such as the development of new techniques or instruments. The perfection of the microscope by Leeuwenhoek was important for Pasteur's work in bacteriology and Metchnikoff's studies on phagocytosis. The introduction of chemical methods played a major role in Landsteiner's fundamental studies on immunologic specificity. Later, the quantitative precipitation method described by Heidelberger and Kendall (1935) was the most important factor in the development of modern immunochemistry. The establishment of physical or physicochemical methods such as ultracentrifugation (Svedberg and Pedersen, 1939), ultrafiltration through membranes of graded pore sizes (Elford, Grabar, 1930–1935), electrophoresis in liq-

uid medium (Tiselius, 1937), filtration through absorption columns, particularly through Sephadex and similar materials (Porath, 1950), electron microscopy, radioactive labeling, immunofluorescence, and many other advances have made possible the discovery of entire new fields of study.

Immunologic Methods

Among the purely immunologic methods still in original or modified form we may mention complement fixation (Bordet and Gengou, 1901), passive hemagglutination (Boyden, 1951), rosette formation (Biozzi and Zaalberg, 1964), plaque formation by immunocompetent cells in agar gel (Jerne, Henry, and Nordin, 1963), and the use of antibodies or antigens labeled by fluorescent compounds (Coons, 1942), by enzymes (Avrameas and Uriel, 1966), and by radioactive elements (Yalow and Berson, 1960). Important contributions were made possible by the use of precipitation in gelled media (Chapter 17). At the beginning of the century, Bechold facilitated the observation of ring tests (properly called disk tests and first used by Ascoli in the diagnosis of anthrax) by performing them in gels. Oudin demonstrated in 1946–1948 that each antigen-antibody complex formed an independent precipitation band, and established the simple diffusion method which allows quantitative measurement. The double diffusion method in gels, developed independently by Ouchterlony and by Elek (1948), is particularly useful for qualitative comparisons of antigens and antibodies; immunoelectrophoretic analysis in gels (Grabar and Williams, 1952–1953) and its quantitative modification (Ressler, 1960; Laurell, 1967) have made large contributions to immunology and other branches of science (see Chapter 17). In 1975, Milstein and Kohler elaborated the method for the generation of hybridoma cells and of monoclonal antibodies.

Other technical advances that have made possible breakthroughs in the investigation of immunologic phenomena include the development of cell or tissue cultures; techniques for separation of various populations of cells, including the use of specific immunoabsorbents; and the advantage of working with pure inbred strains of animals raised under germ-free or at least pathogen-free conditions. Various modifications and improvements of these different methods will be presented in appropriate chapters of this book.

HISTORY OF IMMUNOLOGIC THEORIES: PERSONAL COMMENTS*

Even this brief review of the origins of immunology would lack perspective if we did not urge the reader to consider how much there is yet to be done. Although a considerable body of knowledge has accumulated, many fundamental mechanisms—eg, the induction of antibody formation and the role of immunocompetent cells in cellular immunity—remain to be clarified. Many of the theories proposed to account for these phenomena contain some assumptions or postulates that, in the author's opinion, are either insufficient or superfluous. Since the earliest days of the study of immunologic phenomena, 2 fundamental postulates have persisted: (1) Immunologic phenomena are considered "defense mechanisms." This postulate is historically quite logical because the first observations were of this kind. (2) Under normal conditions, the organism will not react against its own constituents—a concept to which Ehrlich, as we have already noted, applied the term "horror autotoxicus."

Two main theories have been proposed to explain the formation of antibodies and their great variability and multiplicity: (1) The **information theory** holds that it is the antigen which dictates the specific structure of the antibody (Haurowitz and Breinl, 1930). Since it has been shown that antibodies differ in their amino acid sequences, this theory as originally stated has been abandoned. However, other hypotheses have been brought forward, eg, the view that the antigens acting on nucleic acids modify the information (Haurowitz, 1970). (2) The **genetic theory** holds that the information for synthesis of all possible configurations of antibodies exists in the genome and that specific receptors on immunocompetent cells are normally present, as foreseen by Ehrlich. The existence of immunoglobulins on the surfaces of immunocompetent cells has been proved, but this does not mean that all possible configurations for any possible antigenic specificity are really present under normal conditions. It seems difficult to conceive that the total number of immunocompetent cells would be sufficient to account for the enormous numbers of possible antigenic structures. Nevertheless, it would be difficult to imagine a mechanism that would not depend on genetic information.

The cellular aspects of the same problem of antibody formation as envisaged by Jerne (1955) and Burnet (1957–1959) are encompassed in the **clonal selection theory.** The 2 fundamental postulates mentioned above—ie, the "defense mechanism" concept and the prohibition against reaction to "self" constituents—form the basis of this theory. To explain self-recognition and tolerance for endogenous constituents, it has been suggested that cells capable of reacting against self constituents, which must exist in the developing organism, are eliminated or destroyed as **forbidden clones.** On the other hand, if autoantibodies appear, this can only be an abnormal event, as already asserted by Ehrlich, and must be a consequence of **somatic mutations** of certain cells (Burnet, 1965). Several arguments have been advanced both in support of and in opposition to this theory. The idea that all immunologic phenomena are necessarily a part of the organism's defense apparatus has been abandoned by many authors, whereas somatic mutations and **deletion mechanisms** for certain cells are still often taken into

*This section represents the author's personal views concerning the historical development and relative merits of immunologic theories.—*The Editors.*

consideration. More recently, the existence of both helper and suppressor lymphocytes has been admitted (Gershon, Mitchison), but the mechanisms of their actions are not yet established.

The author considers the 2 fundamental postulates mentioned above unnecessary; in 1947 he proposed a simpler explanation of antibody formation, ie, that antibodies are "transporteurs" of catabolic and metabolic substances. Somatic mutations, being a random phenomenon, may modify some items of genetic information, but this cannot explain the appearance of specific autoantibodies. It is now established that certain autoantibodies are present in normal sera. They may also appear in sera from patients with various diseases and can be induced experimentally in animals. At the Microbiology Congress in Rome in 1953, the author suggested that autoantibodies should be regarded as normal physiologic agents which, by their opsonizing activity, serve to help "clean up" the products of dead cells when large numbers of them have been destroyed. Normally, these products can be eliminated after their degradation by the existing autolytic enzymes, and the corresponding immunocompetent cells are therefore not activated to produce antibodies. In cases of massive destruction due to any cause, these enzymes are inhibited by substrate excess, and cells capable of synthesizing autoantibodies, which persist in the normal organism, are induced to form autoantibodies. Thus, for the author, "self-recognition" is at least partially enzymatic, and the formation of antibodies would be a general physiologic mechanism for the elimination of "self" as well as of "not-self" substances. This mechanism would act as a defense system in some cases by eliminating invaders that could not be completely destroyed by the organism's existing enzyme system.

CONCLUSION

Immunology started with the work of a few talented scientists using simple methods and instruments and has grown rapidly during the last decades. Thousands of publications, the creation of independent immunology societies with thousands of members in most of the developed countries, the appearance of many special journals or reviews, and the organization of independent International Congresses (the first in Washington in 1971, the second in Brighton in 1974, the third in Australia in 1977, the fourth in Paris in 1980, and the fifth in Japan in 1983) are characteristic of this period. Immunology now ranks as an independent branch of science, and we can hope with some justification that the fundamental problems still unsolved will soon yield to the intense investigative efforts now going forward and that new areas of basic and clinical immunology meriting investigation will continue to emerge.

A SHORT CHRONOLOGY OF IMPORTANT ACHIEVEMENTS IN IMMUNOLOGY

1798 Edward Jenner
Cowpox vaccination.

1880 Louis Pasteur
Attenuated vaccines.

1883 Elie I.I. Metchnikoff
Phagocytosis, cellular defense theory.

1888 P.P. Emile Roux and A.E.J. Yersin
Bacterial toxins.

1890 Emil A. von Behring and Shibasaburo Kitasato
Antitoxins, foundation of serotherapy.

1893 Waldemar M.W. Haffkine
First massive vaccinations in India.

1894 Richard F.J. Pfeiffer and Vasily I. Isaeff
Immunologic lysis of microbes; bacteriolysis.

1894 Jules J.B.V. Bordet
Complement and antibody activities in bacteriolysis.

1896 Herbert E. Durham and Max von Gruber
Specific agglutination.

1896 Georges F.I. Widal and Arthur Sicard
Test for the diagnosis of typhoid (Widal test) on the basis of the Gruber-Durham reaction.

1900 Karl Landsteiner
A, B, O blood groups.

1900 Jules J.B.V. Bordet and Octave Gengou
Complement fixation reaction.

1901 Max Neisser and R. Lubowski
Complement deviation. (This was noted independently by Friedrich Wechsberg in the same year and is known as Neisser-Wechsberg phenomenon.)

1902 Charles R. Richet and Paul J. Portier
Anaphylaxis.

1903 Nicolas M. Arthus
Specific necrotic lesions; Arthus phenomenon.

1903 Almroth E. Wright and Stewart R. Douglas
Opsonization reactions.

1905 Clemens P. von Pirquet* and Bela Schick
Serum sickness.

1906 Clemens P. von Pirquet
Introduced term allergie.

1908 W. Magnus
Use of immunologic reaction in plant taxonomy.

1910 Henry H. Dale and George Barger
Isolated histamine from ergot (and from animal intestinal mucosa in 1911).

1910 Henry H. Dale and Patrick Playfair Laidlaw
Demonstrated allergic contraction of muscle by histamine.

*Name also appears as Clemens Peter Pirquet von Cesenatico, but common usage is Clemens von Pirquet.

1910 William Henry Schultz
Schultz-Dale test for anaphylaxis.

1910 [Francis] Peyton Rous
Experimental viral cancer immunology.

1921 Albert L.C. Calmette and Camille Guérin
BCG vaccination. (The vaccine was developed beginning in 1906; it was used experimentally on newborns from 1921 to 1924 and then in mass vaccinations.)

1921 Carl W. Prausnitz and Heinz Küstner
Cutaneous reactions.

1923 Gaston Ramon
Diphtheria toxin modified with formaldehyde to produce "anatoxin" (toxoid).

1928 Gregory Shwartzman
Necrotic reactions; Shwartzman phenomenon.

1930 Friedrich Breinl and Felix Haurowitz
Template theory of antibody formation.

1935 Alexandre Besredka
Local immunity; oral immunizations.

1935–36 Michael Heidelberger and Forrest E. Kendall
Pure antibodies; quantitative precipitin reactions.

1936–38 Valy Menkin
Leucotaxine.

1938 Arne Wilhelm Tiselius and Elvin A. Kabat
Demonstrated that antibodies are γ-globulins.

1942 Albert H. Coons et al
Fluorescein labeling; immunofluorescence.

1942 Jules T. Freund
Adjuvant.

1942 Lloyd D. Felton
Immunologic unresponsiveness.

1942 Karl Landsteiner and Merrill W. Chase
Cellular transfer of sensitivity in guinea pigs. (The investigators had been studying delayed hypersensitivity since the 1930s.)

1944 Peter Brian Medawar and Frank Macfarlane Burnet
Theory of acquired immunologic tolerance.

1945 Robin R.A. Coombs, R.R. Race, and A.E. Mourant
Antiglobulin test for incomplete Rh antibodies.

1946 Jacques Oudin
Precipitin reaction in gels.

1947 Pierre Grabar
Theory of "globulines transporteurs."

1948 Orjan Ouchterlony and Stephen D. Elek
Double diffusion (of antigens and antibodies) in gels.

1948 Astrid E. Fagraeus
Antibodies formed in plasma cells.

1948–49 Elvin A. Kabat, W.T.J. Morgan, W.M. Watkins et al
Structure of ABO blood group antigens.

1952 Ogdon Carr Bruton
Agammaglobulinemia described in humans.

1952 James F. Riley and Geoffry B. West
Histamine in mast cells.

1953 Pierre Grabar and C.A. Williams
Immunoelectrophoretic analysis; heterogeneity of immunoglobulins.

1955–57 Niels K. Jerne and Frank Macfarlane Burnet
Clonal selection theory; discovery of human immunodeficiencies.

1956 Ernest Witebsky and Noel R. Rose
Induction of autoimmunity in animals.

1957 H. Hugh Fudenberg and Henry G. Kunkel
Macroglobulins with antibody activity (eg, cold agglutinins, rheumatoid factor).

1958 J. Dausset, F. Rapaport
Histocompatibility antigens on leukocytes.

1959 R.R. Porter, Gerald M. Edelman, and Alfred Nisonoff
Structure and formation of antibody molecules.

1960 R. Yalow and S.A. Berson
Radioactive labeling.

1966 S. Avrameas and J. Urieland P. Nakane and G. Pierce
Labeling by enzymes.

1966 H.N. Claman et al
Cooperation of B and T cells.

1970 Y.S. Tatarinov and V.N. Masyakevich
Trophoblast-specific β_1-glycoprotein. Detection of pregnancy.

1975 C. Milstein and G.J.F. Köhler
Hybridoma cells and monoclonal antibodies.

NOBEL PRIZE WINNERS IN IMMUNOLOGY

1901 Emil von Behring for his work on serum therapy, particularly application against diphtheria.

1908 Paul Ehrlich for his work on fundamental immunology, and Elie Metchnikoff for his discovery of phagocytosis.

1913 Charles Robert Richet for his discovery of anaphylaxis.

1920 (Prize for 1919) Jules Bordet for his discoveries in immunology, particularly complement.

1928 Charles Jules Henri Nicolle for his work on typhus.

1930 Karl Landsteiner for the discovery of human blood groups.

1960 Frank Macfarlane Burnet and Peter Brian Medawar for the discovery of acquired immunologic tolerance.

1972 Gerald Maurice Edelman and Rodney Robert Porter for their discoveries concerning the chemical structure of antibodies.

1977 Rosalyn Yalow for the development of radioimmunoassays of peptide hormones.

1980 Jean Dausset and George Davis Snell for their

discoveries on the histocompatibility antigens on human and animal cells, and Baruj Benacerraf for his work on the genetic control of immune responses.

1984 Georges J.F. Köhler and Cesar Milstein for their description of somatic cell hybridization as a technique for the production of monoclonal antibodies. Niels K. Jerne for his description of the idiotype network.

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The Development of Cellular Immunology: 1960–1985

2

Henry N. Claman, MD

In 1960, the emphasis in immunology was on antibodies—their structure, the cellular basis of their production, and their measurement by serologic techniques. Porter, Edelman, Kunkel, and others had demonstrated that immunoglobulin molecules were heterodimers of heavy and light chains with both constant and variable amino acid sequences and that the specific antigen-binding property of the antibody molecule was a function of its tertiary structure. The *cellular* features of the immune response were not as well understood. The phenomenon of delayed hypersensitivity had been studied for years, but progress in clarification of its mechanism was slow. This was due in part to the absence of quantitative in vitro tests but perhaps in greater part to 3 widely held beliefs, all since shown to be unfounded: (1) that small lymphocytes are end-stage cells which do not divide, (2) that lymphocytes are short-lived, and (3) that mice do not develop delayed hypersensitivity.

THE CLONAL SELECTION THEORY

This was the setting in which a major change in emphasis in immunologic research occurred. The clonal selection theory of antibody production was elaborated independently by Burnet and by Talmage in 1957–1960. The 2 main tenets of the theory were (1) that a given lymphocyte and its progeny—a clone—made antibodies capable of binding to only a limited number of antigens (probably just one); and (2) that the specificity of the clone antedated any experience with the antigen. Thus, the developed immune system contained a full repertoire of lymphocytes, each *precommitted* to a single antigen. Specific antigen stimulated the growth of the unique clone able to make the corresponding antibody, but other clones with different specificities were unaffected. This was a radical change from the "instructionist" concept of antibody formation, which held that a cell could produce any antibody, the specific antibody produced being dictated by the antigen. Furthermore, the clonal selection theory focused attention on the cell—the lymphocyte—rather than on its product—the antibody. The theory was in effect "cellular darwinism" in that it posited the genesis by random mutation of diverse species (clones of lymphocytes) followed by selection of a particular clone by antigen.

The clonal selection theory was vigorously resisted. By what mechanism did the preprogrammed clones arise? And could the genome afford to "waste" an abundance of DNA on variable immunoglobulin regions just for the production of antibody with a single specificity?

THE TWO UNIVERSES OF THE IMMUNE RESPONSE

A number of experimental findings greatly advanced the new field of cellular immunology. At the cellular level, the chance discovery of the mitogenic properties of phytohemagglutinin showed that lymphocytes were not end-stage cells. The plaque-forming technique of Jerne and Nordin made it easy to count single antibody-forming cells, which in turn made it possible to stimulate a primary antibody response in vitro—a long-sought research goal in itself. At the level of the whole organism, a major conceptual advance was the delineation of what have been called the "two universes"—cellular and humoral—of the immune response. Delayed hypersensitivity was a cellular response that could be transferred from sensitized animals to nonsensitized recipients by lymphoid cells but not by serum. Humoral (antibody-mediated) reactions, however, could be transferred via serum from immunized donors. Two investigators then serendipitously showed that the 2 universes were *anatomically* as well as *functionally* distinct. Glick and others found that early removal of the chick bursa of Fabricius impaired antibody formation but not delayed hypersensitivity. Moreover, Miller discovered that neonatally thymectomized mice could not reject skin allografts normally. (Such mice also had impaired antibody formation, as discussed below.) Thus began "the golden age of thymology," when it became clear that the thymus was the organ responsible for the development and maintenance of most lymphoid tissue and for the integrity of the immunologic response. Good and other clinical investigators were reporting "experiments of nature" in patients with specific defects in immune mechanisms. Examples were naturally occurring cases of congenital X-linked hypogammaglobulinemia (Bruton) and thymic aplasia (DiGeorge), which were considered analogous to the experimental models of the bursectomized chick and

the neonatally thymectomized mouse, respectively. In rare instances, defects were reported in *both* universes, as in cases of Swiss agammaglobulinemia (now called severe combined immunodeficiency). These clinical reports demonstrated that what was found in the laboratory in mice and other experimental animals was relevant to human health and disease.

CELL COOPERATION IN THE ANTIBODY RESPONSE

By 1965, it was known that the thymus controlled most aspects of antibody production, but the mechanism of control was obscure. It was clear that the thymus itself did not produce antibodies, perhaps because a blood-thymus barrier blocked antigen access to thymocytes. An effort was made to penetrate this barrier—if one existed—by transferring dispersed thymic cells to lethally irradiated recipient mice which were then injected with sheep red blood cell (SRBC) antigen. It was found that although transferred *spleen* cells could make antibody, transferred thymocytes could not; but that when bone marrow cells were transferred with thymocytes and the recipients immunized with SRBC, abundant amounts of antibody were produced. Subsequently, Mitchell and Miller proved that in this model, *bone marrow cells* contained the antibody-producing cells.

Further investigation showed that the cells capable of making antibody had immunoglobulin on their surfaces and that this immunoglobulin was not only the clone's receptor for the appropriate antigen but a sample of the antibody which would be produced when that clone of cells was appropriately stimulated. In contrast, thymus-derived cells could react with antigen but did not produce antibodies. Thymocytes had a different molecule on their surfaces, identified by a mouse alloantiserum called anti-theta (now anti-Thy). Experiments by Raff, Mitchison, and others showed that theta-bearing cells "helped" immunoglobulin-bearing cells make antibody. The antigen bridge model of thymus-marrow complementation envisaged the thymus-derived cell reacting with one portion of the antigen, a carrierlike determinant linked to a bone marrow-derived cell combining with another portion of the antigen, a haptenlike determinant. This was the origin of the terms T (*t*hymus-derived) helper cells and B (*b*one marrow-derived) antibody-forming cells (AFC). Thus too began the reductionist trend in cellular immunology, whereby morphologically indistinguishable cell populations were divided into separate classes with antisera to cell surface markers (eg, anti-immunoglobulin for B cells and anti-Thy for T cells).

It was equally—or more—important that subpopulations of lymphocytes bearing different cell surface markers were *functionally* distinct as well. The designation of morphologically identical lymphocytes as T cells or B cells was regarded as unnecessarily complicated by those who held to the principle of Occam's razor—that entities should not be multiplied without necessity. The Occam principle was further affronted by Mosier's demonstration that antibody formation required *three* entities: 2 lymphoid cells (T and B) and a nonlymphoid accessory or antigen-presenting cell (APC) most frequently identified as a reticuloendothelial cell with macrophage or dendritic cell characteristics. Thus, the hallmark of immunology became **heterogeneity**. Not only was there heterogeneity in the cellular machinery—it was present in the antibody products as well, because immunoglobulins were being divided into isotype classes (IgM, IgG, IgA, IgD, IgE) as well as subclasses (IgG1, IgG2, etc). This heterogeneity of structure and function is not surprising if one remembers that an effective immune system must react to a formidable array of foreign stimuli, including inert chemicals as well as living pathogens large and small.

In 1970, Gershon discovered another functional subclass of lymphocytes—the suppressor cells. These proved to be antigen-specific T lymphocytes with the capacity to down-regulate both delayed hypersensitivity and antibody production by another series of cell interactions. Alloantisera were derived that identified the T helper and T suppressor subsets in mice and in humans. It became accepted that the net effect of an immunologic perturbation represented the relative balance between helper and suppressor influences.

TRANSPLANTATION IMMUNITY & TOLERANCE

What is now called transplantation immunobiology had been an active field of research in the 1940s and 1950s. The "laws of transplantation genetics" developed by Medawar and colleagues were based on earlier studies by Gorer, O'Gorman, and Snell, who transplanted tumor tissues and proved that graft rejection was an immunologic phenomenon. Mitchison in 1955 showed that transplantation immunity was a function of the lymphoid cells. All of these studies benefited from the work of Snell (who later received the Nobel prize) and others, who developed inbred strains of animals, particularly mice. However, the negative side of immunity—ie, tolerance—was always closely associated with studies of the immune state. A dazzling experiment by Billingham, Brent, and Medawar in 1953 showed that specific transplantation tolerance could be induced in embryonic or newborn mice by transfer of adult cells. The rationale for this approach was clearly based on Burnet's clonal selection theory and the relative immunologic incompetence of the immature mammal. This elegant experiment left many details still not fully explained, but it remains the conceptual starting point for the study of host-versus-graft reactions. Soon afterward, the converse phenomenon—graft-versus-host reactions—were studied in elaborate detail.

While the mechanisms of self-tolerance and acquired tolerance are still not clear, Nossal provided useful studies of the chemical requirements and cellu-

lar bases of tolerance. Chiller and Weigle showed that tolerance could exist at the T or B cell level—or at both levels. Investigations of tolerance of the immune system would have to take into account the possible involvement of suppressor cells as well as intrinsic unresponsiveness in T or B cells.

IMMUNOREGULATION & THE NETWORK HYPOTHESIS

Great emphasis during this time was placed on how the immune response was *regulated*. Immunoregulating mechanisms may be broadly conceived as proceeding along 2 pathways: antigen-specific and antigen-nonspecific. Specific immunoregulatory mechanisms were the focus of the **network hypothesis** of Jerne, who proposed that for each antigen receptor or idiotype (a T cell receptor or an immunoglobulin on a B cell) there was a corresponding clone of cells bearing a complementary anti-idiotypic receptor (see Chapter 11). Jerne postulated that antigen A would stimulate the expansion of an antigen-reactive clone bearing the idiotype anti-A and that this clonal expansion would then be sensed by another clone bearing the anti-idiotype complementary to anti-A, which in turn would be stimulated to expand. In the B cell system, antigen A would stimulate its antibody, anti-A, and this in turn would cause production of the anti-idiotypic antibody anti-(anti-A). These antibodies have been called antibody-1 (conventional antibody, anti-A) and antibody-2 (anti-anti-A, the anti-idiotypic antibody). Such antibodies have in fact been demonstrated to exist following immunization of animals with a single antigen. One of Jerne's contributions was the notion that anti-idiotypic reactions would be down-regulatory and give rise to negative feedback mechanisms that would inhibit exaggerated responses of the immune system. Indeed, many anti-idiotypic antibodies are known down-modulators. Network mechanisms also influence T cell reactions, as shown by tolerance models of cell-mediated immunity in which inhibition is imposed along a sequence of idiotype-anti-idiotype suppressor T cell cascades.

The attention that was now directed toward the antigen-nonspecific features of the immune response represented a basic shift in emphasis because until this time one of the 2 hallmarks of the immune response had been **specificity** (the other was **memory**). If a biologic response was not antigen-specific, it probably was neither immunologic nor important. Experiments using nonspecific adjuvants were considered "inelegant." All this changed with the discovery of a variety of lymphokines and cytokines, including interleukins-1, -2, and -3, as well as various interferons and lymphocyte growth and differentiation factors. It became clear that although specific antigenic triggers could stimulate T and B cell clonal responses, the magnitude of the eventual response depended upon the activity of these antigen-nonspecific modulatory cytokines. These putative substances entered the literature in complex array, each bearing aloft its acronymic gonfalon (MIF, MAF, SMAF, etc) and each based on a specific bioassay procedure—yet none of them holding a secure place in the intricate regulatory scheme. The situation was clarified in the 1980s by the work of nomenclature committees and by workers who were able to clone genes for the cytokines and develop recombinant cytokine molecules and monoclonal antibodies to them.

These matters are discussed in greater detail in Chapter 8.

IMMUNOGENETICS & MOLECULAR BIOLOGY

The discovery that the genes controlled the immune responses to specific antigens was a critical development in modern immunology. It was found that certain inbred strains of animals ("responders") could make antibody to a given antigen whereas other "nonresponder" strains could not. Strain S might be a responder for antigen X and a nonresponder for antigen Y, whereas strain T animals might respond to Y but not to X. An even more striking finding was that responder or nonresponder status was a function of the major histocompatibility complex (MHC) (see Chapter 6). The MHC section of the genome was being explored at both the gene level and the gene product level and had been divided into class I, II, and III regions. T cell recognition of antigen was shown to be a more complex phenomenon than B cell recognition. While B cell recognition of antigen occurred at its surface immunoglobulin receptor, helper T cells could be activated only by antigen presented to it in the context of class II gene products. The genetic control of immune responses thus turned on the nature of the T cell receptor for antigen. The nature of the T cell receptor was the subject of vigorous international debate for about 10 years. Was the receptor an immunoglobulin—as had been demonstrated in the case of B cells—or was it some completely different molecule? The answer proved to be that the T cell receptor was "immunoglobulin*like*," meaning that the constitutent chains of the T cell receptor molecules that recognized antigen plus class II MHC could be divided into constant and variable regions. It became obvious also that the molecules comprising the T cell receptor were different from those of which serum antibodies were constituted and were derived from distinct genes. Thus, the antigen recognition molecules used by T cells and B cells were similar but not identical, and they probably had a common evolutionary origin.

In the chronology of molecular immunologic research, studies of immunoglobulin structure and the genetic mechanisms of antibody diversity actually preceded discovery of the T cell receptor. Indeed, the latter advance was made possible by work that had been going forward since the early 1970s. The observation that different antibody molecules might have the same constant regions but different variable regions discred-

ited the "one gene, one polypeptide chain" concept. It thus became clear that the assembly of a single immunoglobulin molecule called for a complex scheme of gene splicing involving V, J, D, and C segments (see Chapter 5). Moreover, controversy continued about the nature of the events involved in generating the more than 1 million distinct clones of T and B cells necessary to react to the more than 1 million different antigens the immune system must contend with. Was it based on selection and rearrangement of genomic material, or did somatic mutation account for T and B cell development during ontogeny? The ultimate answer, as is often the case, is that both processes contributed.

The developments summarized in the preceding paragraphs would not have been possible without methodologic advances such as rapid DNA sequencing, gene and cell cloning, and the invention of monoclonal antibodies utilizing somatic cell hybridization. Monoclonal antibody production coupled with the development of T cell hybridomas and cloned lines of specific T and B cells enabled cellular immunologists to work with homogeneous cell populations in the way biochemists worked with purified proteins. A recent refinement of this approach has been to use cloned T cells and cloned EBV-infected B cells from a single donor that reacted to a single antigen. The conclusion reached is that the antigen bridge may not account for T cell/B cell collaboration—it may in fact be the case that each cell recognizes similar antigenic determinants.

THE BROADER REACHES OF IMMUNOLOGY

This brief account of the development of cellular immunology has necessarily left unsung the outstanding contributions made in other fields of biologic investigation. All of them are given appropriate space in other chapters of this book. They can be listed here as follows:

(1) Studies of the classic and alternative **complement pathways** and their relationship to inflammation, autoimmunity, and host defenses. (See Chapter 10.)

(2) The identification of IgE as the immunoglobulin class responsible for **anaphylactic allergies** and elucidation of the chemical mediators released during anaphylactic reactions. (See Chapter 15.)

(3) Research into the mechanisms of immunologic defense against infections—in particular the development of **immunoparasitology.** The rational development of vaccine therapy also promises early benefits. (See Chapter 13.)

(4) Advances in understanding the biology of **malignant neoplastic disease,** including the therapeutic use of specific monoclonal antibodies and nonspecific immunoadjuvants and immunosuppressants. (See Chapter 14.)

(5) Extension of the techniques of organ transplantation to include not only kidneys but also heart, liver, bone marrow, pancreatic islet cells, skin, and even neural tissue. (See Chapter 23.) With bone marrow transplantation came the recognition that graft-versus-host reactions were not just curiosities to be gawked at in the immunologist's laboratory but powerful and often dangerous clinical reactions. A positive result was that the study of these reactions provided models for the study of autoimmunity.

(6) Studies of the nature and pathogenesis of congenital and acquired immunodeficiency states (including, most recently, AIDS) and of ways they might be treated or prevented. (See Chapter 21.)

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3 Immunogenicity & Antigenic Specificity

Joel W. Goodman, PhD

IMMUNOGENS

Immunogenicity

Immunogenicity is a property of substances that can induce a detectable immune response (humoral, cellular, or, most commonly, both) when introduced into an animal. Such substances are called immunogens or **antigens.**

Chemical Nature of Immunogens

The most potent immunogens are macromolecular proteins, but polysaccharides, synthetic polypeptides, and other synthetic polymers such as polyvinylpyrrolidone are immunogenic under appropriate conditions (see below). Although pure nucleic acids have not been shown to be immunogenic, antibodies that react with nucleic acids may be induced by immunization with nucleoproteins. Such antibodies appear spontaneously in the serum of patients with systemic lupus erythematosus (see Chapter 21).

Requirements for Immunogenicity

Immunogenicity is not an inherent property of a molecule, as are its physicochemical characteristics, but is operationally dependent on the experimental conditions of the system. These include the antigen, the mode of immunization, the organism being immunized, and the sensitivity of the methods used to detect a response. The factors that confer immunogenicity on molecules are complex and incompletely understood, but it is known that certain conditions must be satisfied in order for a molecule to be immunogenic.

A. Foreignness: The immune system somehow discriminates between "self" and "nonself," so that only molecules that are foreign to the circulation of the animal are normally immunogenic. Thus, albumin isolated from the serum of a rabbit and injected back into the same or another rabbit will not generate the formation of antibody. Yet the same protein injected into any other higher vertebrate animal is likely to evoke substantial amounts of antibody depending on the dose of antigen and the route and frequency of injection.

B. Molecular Size: Extremely small molecules such as amino acids or monosaccharides are not immunogenic, and it is generally accepted that a certain minimum size is necessary for immunogenicity. However, there is no specific threshold below which all substances are inert and above which all are active, but rather a gradient of immunogenicity with molecular size. In a few instances, substances with molecular weights of less than 1000 have proved to be immunogenic, but as a general rule molecules smaller than molecular weight 10,000 are only weakly immunogenic or not immunogenic at all. The most potent immunogens are macromolecular proteins with molecular weights greater than 100,000.

C. Chemical Complexity: A molecule must possess a certain degree of chemical complexity in order to be immunogenic. The principle has been illustrated very clearly with synthetic polypeptides. Homopolymers consisting of repeating units of a single amino acid are poor immunogens regardless of size, whereas copolymers of 2 or—even better—3 amino acids may be quite active. Once again, it is difficult to establish a definite threshold, and the general rule is that immunogenicity increases with structural complexity. Aromatic amino acids contribute more to immunogenicity than nonaromatic residues, since relatively simple random polypeptides containing tyrosine are better antigens than the same polymers without tyrosine, and immunogenicity is proportionate to the tyrosine content of the molecule. Also, the attachment of tyrosine chains to the weak immunogen gelatin, which is poor in aromatic amino acids, markedly enhances its immunogenicity.

D. Genetic Constitution of the Animal: The ability to respond to a particular antigen varies with genetic makeup. It has been known for some time that pure polysaccharides are immunogenic when injected into mice and humans but not when injected into guinea pigs. Much additional information has accrued from the use of inbred strains of animals. As one of many examples, strain 2 guinea pigs respond readily in an easily detectable manner to poly-L-lysine, whereas strain 13 guinea pigs do not. The ability to respond is inherited as an autosomal dominant trait. Many similar cases have been described, and the genetic control of the immune response is currently one of the most active areas of investigation in biology (see Chapter 6).

E. Method of Antigen Administration: Whether an antigen will induce an immune response depends on the dose and the mode of administration. A quantity

of antigen which is ineffective when injected intravenously may evoke a copious antibody response if injected subcutaneously in adjuvant. In general, once the threshold is exceeded, increasing doses lead to increasing—but less than proportionate— responses. However, excessive doses may not only fail to stimulate antibody formation; they can establish a state of specific unresponsiveness.

ANTIGENIC DETERMINANTS

Although strong immunogens are large molecules, only restricted portions of them are involved in actual binding with antibody combining sites. Such areas, which determine the specificity of antigen-antibody reactions, are designated antigenic determinants. The number of distinct determinants on an antigen molecule usually varies with its size and chemical complexity. Valence estimates have been made on the basis of the number of antibody molecules bound per molecule of antigen. Such measurements provide minimum values, since steric hindrance may prevent simultaneous occupation of all sites. Furthermore, antibody populations from different animals are likely to vary in specificity, and variations occur also in those of a single individual at different points in time. This means that antibodies specific for all determinants of an antigen molecule may not be present in a particular antiserum. Typical results using this approach give about 5 antigenic determinants for hen egg albumin (MW 42,000) and as many as 40 for thyroglobulin (MW 700,000).

Haptens

Much of our understanding of the specificity of antigen-antibody reactions derives from the pioneering studies of Karl Landsteiner in the early years of the 20th century with small, chemically defined substances which are not immunogenic but can react with antibodies of appropriate specificity. They are called haptens, from the Greek word *haptein*, "to fasten." Landsteiner covalently coupled the diazonium derivatives of a wide variety of aromatic amines to the lysine, tyrosine, and histidine residues of immunogenic proteins (Fig 3–1). The conjugated proteins raised antibody specific for the azo substituents, demonstrated by the capacity of the free hapten to bind antibody. The conjugated hapten therefore behaves like a partial or complete antigenic determinant. The total determinant may include amino acids in the protein to which the hapten is linked. The protein, called the **carrier,** has its set of native or integral determinants as well as the new determinants introduced by the conjugated hapten (Fig 3–2).

Although most haptens are small molecules, macromolecules may also function as haptens. The definition is based not on size but on immunogenicity.

The use of hapten-protein conjugates has spotlighted the remarkable diversity of immune mechanisms as well as the exquisite structural specificity of antigen-antibody reactions. Virtually any chemical entity may serve as an antigenic determinant if coupled to a suitably immunogenic carrier. Even antibodies with specificity for metal ions have been produced in this way.

Landsteiner's studies showed that antibody could distinguish between structurally similar haptens. In one series of experiments, antibodies raised to *m*-aminobenzenesulfonate were tested for their ability to bind with other isomers of the homologous hapten and related molecules in which the sulfonate group was replaced by arsonate or carboxylate groups (Table 3–1). As expected, the strongest reaction occurred with the homologous hapten. The compound with the sulfonate group in the *ortho* position was somewhat poorer than the *meta* isomer but distinctly better than the *para* isomer. The substitution of arsonate for sulfonate resulted in very weak binding with antibody. Although both substituents are negatively charged and have a tetrahedral structure, the arsonate group is bulkier because of the larger size of the arsenic atom and the additional hydrogen atom. The benzoate derivatives are also negatively charged, but the carboxylate ion has a

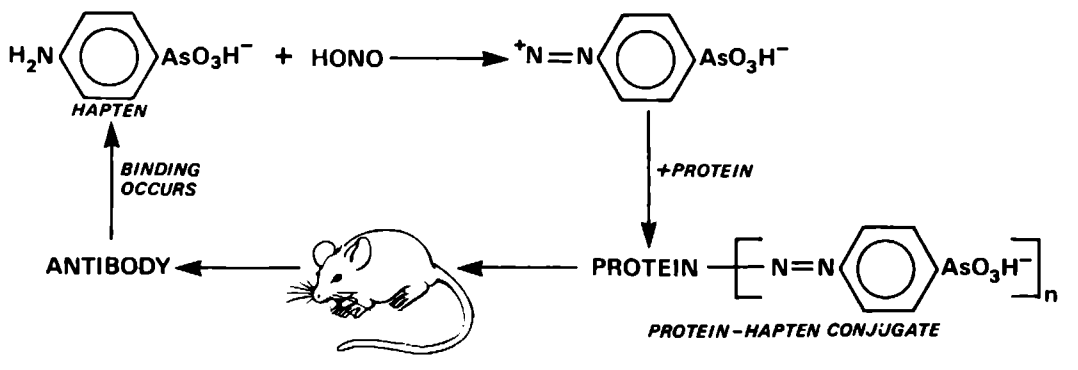


Figure 3–1. The preparation of hapten-protein conjugates and their capacity to induce the formation of antihapten antibody to the azophenylarsonate group in this example.

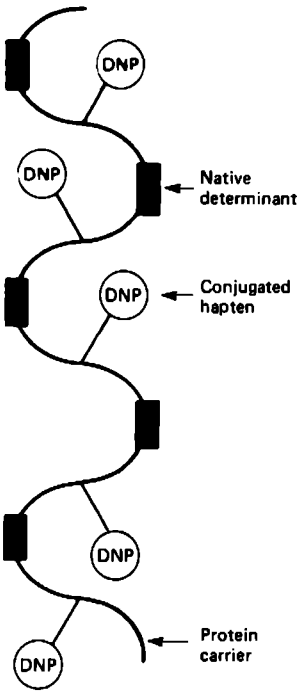


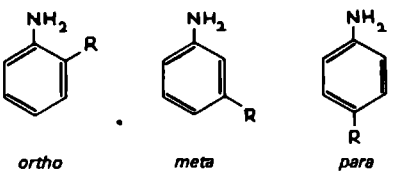
Figure 3–2. Diagrammatic illustration of a hapten-protein conjugate. The protein has several native or integral antigenic determinants denoted by thickened areas. The conjugated dinitrophenyl (DNP) hapten introduces new antigenic determinants.

planar rather than tetrahedral 3-dimensional configuration and shows even less affinity for the antisulfonate antibody.

The reaction of antibody with an antigen or hapten other than the one that induced its formation is called a **cross-reaction.** Thus, the reaction of anti-*m*-aminobenzenesulfonate with any of the other compounds in Table 3–1 is a cross-reaction. Cross-reactions almost invariably have a lower binding affinity than homologous reactions between antibody and its inducing antigen.

Studies of this kind have shown that antibody recognizes the overall 3-dimensional shape of the antigenic determinant group rather than any specific chemical property such as ionic charge. It is believed that antigenic determinants and antibody combining sites possess a structural complementariness which may be figuratively visualized as a "lock-and-key" arrangement (Fig 3–3). The electron cloud box of the antibody site is contoured to match that of the antigenic determinant, with the affinity of binding directly proportionate to the closeness of fit. The startling diversity of the antibody response is perhaps more comprehensible if antibody specificity is viewed as directed against a molecular shape rather than a particular chemical structure.

Table 3–1. Effect of variation in hapten structure on strength of binding to *m*-aminobenzenesulfonate antibodies.



ortho *meta* *para*

	ortho	*meta*	*para* isomers
R = sulfonate	++	+++	±
R = arsonate	–	+	–
R = carboxylate	–	±	–

Strength of binding is graded from negative (–) to very strong (+++). (From Landsteiner K, van der Scheer J: On cross reactions of immune sera to azoproteins. *J Exp Med* 1936;63:325.)

Size & Location of Antigenic Determinants

Antibody complementarity is directed against limited parts of the antigen molecule. Numerous studies using homopolymers of sugars or amino acids or multichain polymer-protein conjugates indicate that an antigenic determinant is of the order of 4–6 amino acid or sugar residues (Fig 3–3). The weight of evidence also indicates that the entire exposed surface of a protein may be antigenic. Therefore, large proteins express many potential determinants. However, a given individual will make antibodies against only a small subset of the total. For example, as noted above, a given antiserum to hen egg albumin has specificity for no more than about 5 determinants. Since the total number when comparing different antisera is much

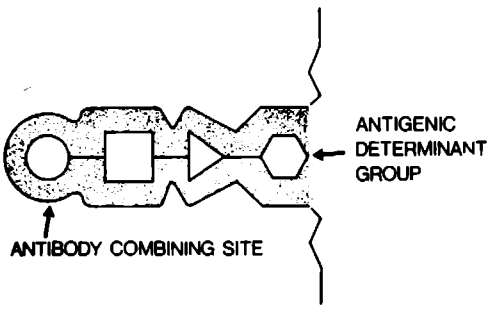


Figure 3–3. A view of the "lock-and-key" complementariness between an antigenic determinant group and an antibody combining site. The determinant can be considered to be composed of discrete subunits, which may be amino acids in a peptide chain or sugars in a saccharide chain. The antibody combining site is then composed of subsites, each of which can accommodate a discrete subunit of the antigenic determinant. (Reproduced, with permission, from Goodman JW: Antigenic determinants and antibody combining sites. In: *The Antigens*. Vol 3. Sela M [editor]. Academic Press, 1975.)

greater, there is obviously a selection of potential determinants in any given situation.

A cardinal factor in the selection of determinants is exposure to the aqueous milieu and therefore to the immune apparatus. The terminal side chains of polysaccharides represent the most potent determinant regions of that class of compounds. The principle has been demonstrated most vividly with multichain synthetic polypeptides having sequences of alanine on the inside and tyrosine-glutamic acid closer to the backbone, or the reverse (Fig 3–4). Antibodies to the former were largely alanine-specific, whereas the latter evoked antibodies with a predominant specificity for tyrosine-glutamic acid sequences. The most exposed region was preferred as the determinant in each instance. The same is generally true for globular proteins. A feature of proteins that correlates well with accessibility and has had predictive value for identifying determinants is the **hydrophilicity** of local regions within the protein. The greater the average hydrophilicity, the higher the likelihood that the region will be antigenic.

In addition to accessibility, which is an intrinsic feature of the antigen, host factors play important roles in determinant selection and probably account for the different specificity patterns in antisera from different individuals. A large body of evidence attests to the genetic control of antibody specificity to a given antigen. Some of the earliest evidence accrued from a comparison of the specificity of anti-insulin antibodies from strain 2 and strain 13 guinea pigs, which are uniformly directed against opposite ends of the insulin molecule. Many other examples have been found in the responses of inbred strains of mice (see Chapter 6). Outbred populations are more difficult to study, but the principle that genetic makeup strongly influences determinant selection has been clearly established.

Immunodominance

Given a particular determinant, which may be the size of a tetrapeptide, the amino acid subunits of that determinant will contribute unequally to binding with antibody. The degree of the influence on reactivity is a measure of the immunodominance of the component.

Antibody specificity may be directed against conformational or sequential features of antigens. The former usually holds for globular proteins and helical structures. For example, polymers of the tripeptide L–tyrosyl–L–alanyl–L–glutamic acid form an α-helix under physiologic conditions. The same tripeptide can be attached to a branched synthetic polypeptide (Fig 3–5). The tripeptide itself does not possess an ordered configuration. Antibodies to the 2 polymers do not cross-react, and the tripeptide binds antibodies produced against the branched polymer but not those made against the helical polymer. The immunodominant element of the helical polymer is its conformation. Antiserum against human hemoglobin A_1 com-

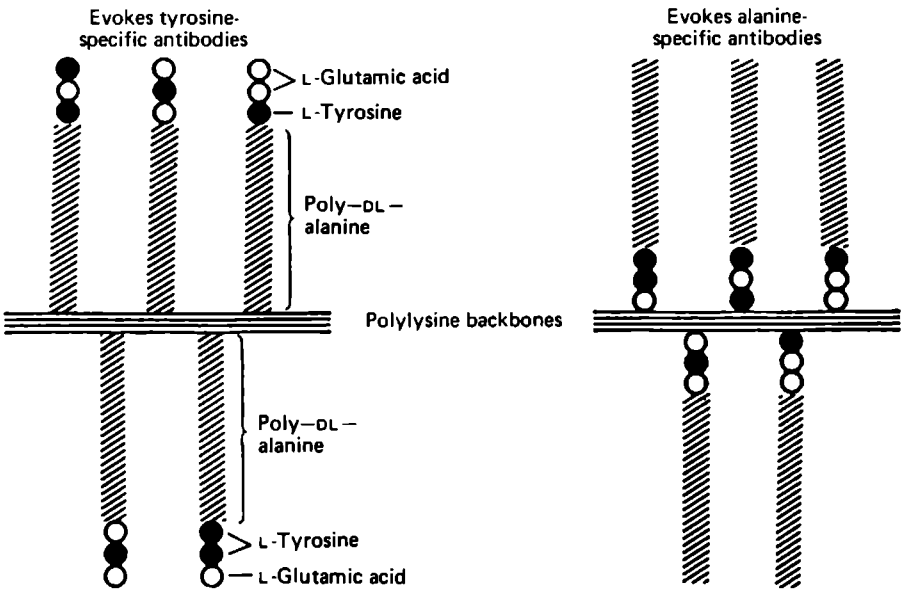


Figure 3–4. ***Left:*** A multichain copolymer in which L-tyrosine and L-glutamic acid residues are attached to multi-poly-DL-alanyl-poly-L-lysine(poly-[Tyr,Glu]-poly-DL-Ala-poly Lys). ***Right:*** Copolymer in which tyrosine and glutamic acid are attached directly to the polylysine backbone with alanine peptides on the ends of the side chains. Horizontal lines: poly-L-lysine; diagonal hatching: poly-DL-alanine; closed circles: L-tyrosine; open circles: L-glutamic acid. (From Sela M: Antigenicity: Some molecular aspects. *Science* 1969;166:1365. Copyright © 1969 by the American Association for the Advancement of Science.)

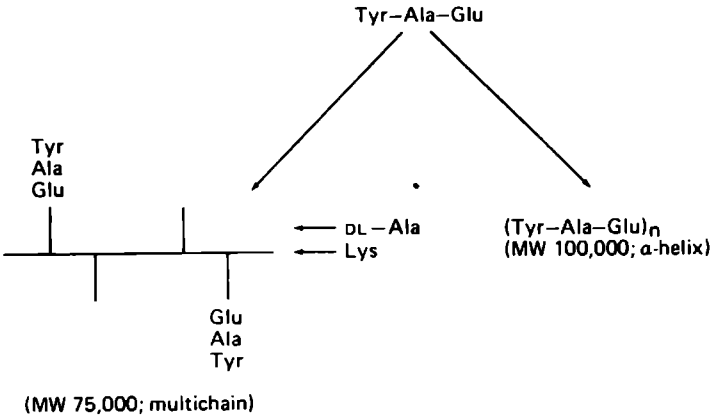


Figure 3–5. A synthetic branched polymer in which peptides of sequence Tyr-Ala-Glu are attached to the amino groups of side chains in multi-poly-DL-alanyl-poly-L-lysine *(left)* and a periodic polymer of the tripeptide Tyr-Ala-Glu *(right)*. (From Sela M: Antigenicity: Some molecular aspects. *Science* 1969;166:1365. Copyright © 1969 by the American Association for the Advancement of Science.)

bines better with the oxygenated form than the reduced form, and this has been attributed to the difference in quaternary structure between the 2 forms. There are many examples of conformation-dependent antibody specificity.

Determinants whose specificity is dictated by the sequence of subunits (amino acids or sugars) within the determinant rather than by the macromolecular superstructure of the antigen molecule are designated **sequential determinants.** In such cases, components of the determinant can act as haptens and bind with antibody, the reaction being demonstrable either directly, by such techniques as equilibrium dialysis or fluorescence quenching, or indirectly, by inhibition of the reaction between antigen and antibody. Sequential determinants may be composed of terminal or internal sequences of macromolecules, or they may be artificially added to carriers, as in the case of the tripeptide Tyr-Ala-Glu. Characterization of the antigenic structure of several proteins has shown that sequential determinants are always localized in hydrophilic regions of the molecule, where exposure to the aqueous environment is maximal.

When the antigenic determinant is a terminal sequence, the terminal residue of the sequence is almost invariably the immunodominant subunit. Again, many examples exist to illustrate this point, which was recognized by Landsteiner when he showed that the terminal amino acid of peptides coupled to a protein carrier exerted a dominant effect on specificity. Goebel made the same observation with glycosides conjugated to protein carriers. In general, then, it may be concluded that all determinants exhibit a gradient of immunodominance. When the determinant is comprised of a terminal sequence, the gradient decreases from the most exposed portion inward.

In addition, antigenic determinants may be continuous or discontinuous. If antibodies bind to a contiguous sequence of amino acids, the determinant is continuous. A discontinuous determinant, on the other hand, is comprised of residues that are separated from one another in the sequence of the protein but are brought into proximity by tertiary folding. There are numerous examples of discontinuous determinants in proteins. Conformational determinants may be continuous or discontinuous, but sequential determinants are always continuous.

IMMUNOGENIC DETERMINANTS

Immunogens are normally large molecules, and immunogenicity is, within limits, a function of molecular size and complexity. A characteristic of immunogens is their capacity to induce cellular immunity mediated by thymus-derived T lymphocytes (see Chapter 7), which haptens are unable to do. It is believed that an immunogen must possess at least 2 determinants in order to stimulate antibody formation, which is the function of another line of lymphocytes, bursa-derived B cells. At least one determinant must be capable of triggering a T cell response. These relationships and the concept of cell cooperation are discussed in greater detail in Chapter 7. Our concern here is with structural determinants of immunogens that interact with T and B lymphocytes. It has not been possible to identify such determinants on large proteins, but studies with small, well-defined immunogens support the interpretation that specificities of the 2 cell types may be directed against different determinants of the antigen molecule.

The pancreatic hormone glucagon consists of only 29 amino acids but is immunogenic. It has been functionally dissected into component determinants which interact with T cells (immunogenic determinants) and with antibody (haptenic determinants). Using isolated

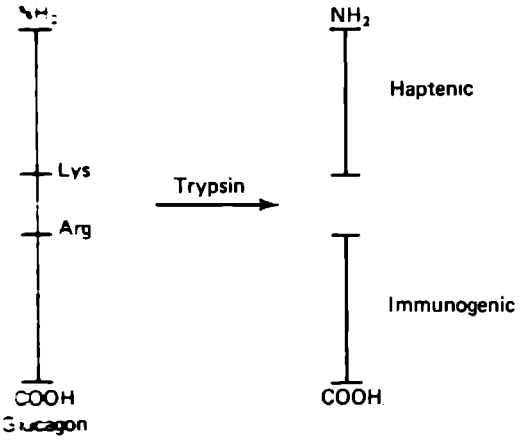


Figure 3–6. The functional dissection of glucagon into immunogenic and haptenic determinants.

tryptic peptides of the hormone, it was found that antibodies recognized a determinant or determinants in the amino terminal part of the molecule, whereas T lymphocytes responded only to the carboxy-terminal fragment (Fig 3–6). The latter was therefore identified as the immunogenic or "carrier" portion of the molecule and the former as the haptenic region.

Several synthetic molecules about the size of a single antigenic determinant induce an almost purely cellular immune response, with little or no antibody production, but are capable of acting as carriers for conjugated haptens in much the same fashion as macromolecular immunogens. One such unideterminant immunogen is the compound L-tyrosine-*p*-azobenzenearsonate (ABA-Tyr). Despite its molecular weight of only 409, ABA-Tyr induces cellular immunity with little or no antibody production in a variety of animal species. A hapten such as the dinitrophenyl group can be coupled to ABA-Tyr through a spacer group (6-aminocaproic acid) to produce a bideterminant or bifunctional immunogen (Fig 3–7). This antigen induces antibody specific for the dinitrophenyl haptenic determinant and cellular immunity directed against the ABA-Tyr immunogenic determinant.

Another example is the response of guinea pigs to poly-L-lysine. Responder animals (strain 2 and a fraction of outbred guinea pigs) develop cellular immunity to polymers as small as the heptapeptide. The bifunctional immunogen α-dintrophenyl-(L-Lys)$_7$ induces antidinitrophenyl antibody responses. Smaller oligomers of lysine do not induce cellular immunity and cannot act as carriers for the dinitrophenyl haptenic determinant.

Experiments with analogs of immunogenic determinants, designed along the lines of Landsteiner's classic studies on the specificity of antihapten antibodies, have shown that cellular (T cell) responses to antigens are as exquisitely specific as antigen-antibody reactions.

Recent findings indicate that in some instances different determinants on a protein antigen may activate different functional subpopulations of T cells (see Chapter 7). For example, different fragments of myelin basic protein induce suppression and immunity in rodents. Immunity is manifested as an autoimmune allergic encephalomyelitis. Animals presensitized with the suppressor-inducing fragment and subsequently challenged with the intact molecule did not develop encephalomyelitis. A determining factor in the selective activation of suppressor or helper T cells by particular determinants appears to be the genetic constitution of the animal. Thus, the same region (though perhaps not the identical determinant) of hen egg lysozyme induces suppression in strain B10 mice but helps in strain B10.A mice. Another example is the induction of suppression or help by a random synthetic copolymer of glutamic acid, alanine, and tyrosine in different inbred strains of mice.

The selective activation of help or suppression is being actively investigated, because it may eventually offer a rationale for manipulating the immune response in humans to such clinically important antigens as histocompatibility antigens, tumor antigens, and allergens.

THYMUS-INDEPENDENT ANTIGENS

A certain type of molecule may be immunogenic without the apparent participation of T lymphocytes. Such molecules appear to be able to directly trigger B lymphocytes (antibody-producing cells). Their characteristic feature is a structure that consists of repeating units. Bacterial polysaccharides and some polymerized proteins are thymus-independent antigens. However, not all repeating unit polymers behave this

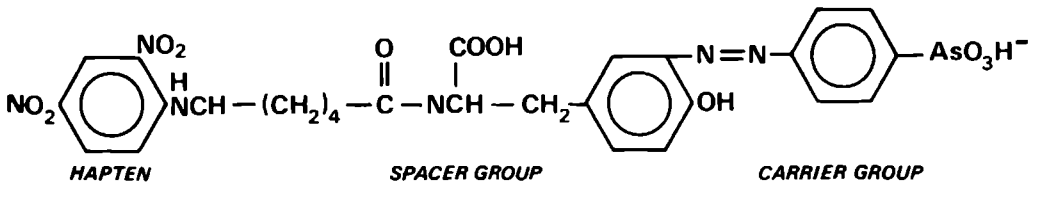


Figure 3–7. The bifunctional antigen dinitrophenyl-6-aminocaproyl-L-tyrosine-*p*-azobenzenearsonate.

way. Poly-L-lysine, for example, is a thymus-dependent antigen in responder guinea pigs despite its simple, repetitive structure.

The mechanism by which thymus-independent antigens act is still unclear, but the immune response to such antigens differs from the response to more typical thymus-dependent antigens in that the antibody produced is largely or exclusively of the IgM class and little or no immunologic memory is engendered. Recent careful analysis of the responses to these antigens indicates that many, if not all, do require some degree of T cell help, although significantly less than that required by conventional thymus-dependent antigens. Therefore, it may be more accurate to consider them as thymus-efficient rather than as thymus-independent antigens.

SYNTHETIC VACCINES

Two promising new approaches to vaccine development have emerged in the modern era of biomedical technology. One is the cloning of genes coding for important surface proteins of infectious agents, with production of large quantities of the desired protein by microorganisms transfected with the gene. The other is the synthesis of short peptides from the known sequences of the proteins and attachment of the peptide haptens to protein carriers, thereby creating "synthetic antigens." This second approach is predicated on the assumption that antibodies induced to short peptides of the order of 6–15 amino acids will react with the homologous sequences in the native proteins. While it is true that antibodies to proteins are often conformation-specific and react weakly or not at all with peptide fragments of the protein, the reverse may not necessarily hold. Indeed, it has been reported by Lerner that antibodies raised to 17 out of 20 synthetic peptides, representing about 75% of the hemagglutinin protein of influenza virus, bound to the virus itself. These findings therefore suggest that a peptide from almost any region of a protein can elicit antibodies which will react with the native molecule, although the affinity of binding may be different for the 2 reactions. The only stringent requirement seems to be that the peptide sequence reside on the surface of the folded protein, where it is accessible to antibody.

Lerner's findings offer promise for the manufacture of synthetic vaccines for use in human and animal prophylaxis. However, an important consideration here is that immunologic memory (anamnesis; see the Appendix) in the response to hapten-carrier conjugates is directed at the carrier as well as the hapten. Since the carriers are different in the synthetic vaccine and the native protein from which the peptide came, an encounter with the infectious agent following immunization with the vaccine should elicit little or no anamnesis unless the peptide also harbors an immunogenic (T helper cell) determinant. However, if the antibody titer raised by the vaccine remains elevated for long periods of time, substantial protection may be provided even in the absence of anamnesis. It is not yet clear how useful synthetic vaccines will prove to be.

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Immunoglobulins I: Structure & Function

4

Joel W Goodman, PhD

The immunoglobulins are the protein molecules that carry antibody activity, ie, the property of specific combination with the substance which elicited their formation (**antigen**). With the possible exception of **natural** antibody, antibodies arise in response to foreign substances introduced into the body. The immunoglobulins comprise a heterogeneous group of proteins that account for approximately 20% of the total plasma proteins. In serum electrophoresis, the majority of the immunoglobulins migrate to the zone designated **γ-globulin,** but significant amounts are also found in the **β-globulin** zone. Different populations of immunoglobulins are also found in varying proportions in extravascular fluids, in exocrine secretions, and on the surface of some lymphocytes. The biologic activities of immunoglobulins can only be understood on the basis of knowledge of their structure, and in this chapter will be described the structure and evolution of immunoglobulin molecules.

BASIC STRUCTURE & TERMINOLOGY

Immunoglobulins are glycoproteins composed of 82–96% polypeptide and 4–18% carbohydrate. The polypeptide component possesses almost all of the biologic properties associated with antibody molecules. Antibodies are bifunctional molecules in that they bind specifically with antigen and also initiate a variety of secondary phenomena, such as complement fixation and histamine release by mast cells, which are independent of their specificity for antigen. Antibody molecules are extremely heterogeneous, as might be expected in view of their enormous diversity with respect to antigen binding and their different biologic activities. This heterogeneity is easily demonstrated by serologic, electrophoretic, and amino acid sequence methods and severely hampered early structural studies.

Two major discoveries ushered in the period of detailed structural study of antibodies. The first was the finding that enzymes and reducing agents could be used to digest or dissociate immunoglobulin molecules into smaller components. The second was the realization that the electrophoretically homogeneous proteins found in serum and urine of patients with multiple myeloma (see Chapter 22) were related to normal immunoglobulins. These myeloma proteins were found to be structurally homogeneous. They are also called monoclonal proteins, since they are synthesized by single clones of malignant plasma cells. A clone here refers collectively to the progeny of a single lymphoid cell.

Our present understanding of immunoglobulin structure is based collectively on studies of monoclonal and normal proteins. The discussion of the details of immunoglobulin structure is introduced with a list of definitions of the relevant terms used here and in Figs 4–1 and 4–2.

List of Definitions

Basic unit (monomer): Each immunoglobulin contains at least one basic unit or monomer comprising 4 polypeptide chains (Fig 4–1).

H and L chains: One pair of identical polypeptide chains contains approximately twice the number of amino acids, or is approximately twice the molecular weight, of the other pair of identical polypeptide chains. The chains of higher molecular weight are designated **heavy (H) chains** (Fig 4–1) and those of lower molecular weight **light (L) chains.**

V and C regions: Each polypeptide chain contains an amino-terminal portion, the **variable (V) region;** and a carboxy-terminal portion, the **constant (C) region.** These terms denote the considerable heterogeneity or variability in the amino acid residues in the V region compared to the C region.

Domains: The polypeptide chains do not exist 3-dimensionally as linear sequences of amino acids but are folded by disulfide bonds into globular regions called domains. The domains in H chains are designated V_H and C_H1, C_H2, C_H3, and C_H4; and those in L chains are designated V_L and C_L.

Antigen-binding site: The part of the antibody molecule that binds antigen is formed by only small numbers of amino acids in the V regions of H and L chains. These amino acids are brought into close relationship by the folding of the V regions.

Fab and Fc fragments: Digestion of an IgG molecule by the enzyme papain produces 2 Fab (antigen-binding) fragments and one Fc (crystallizable) fragment.

Hinge region: The area of the H chains in the C region between the first and second C region domains (C_H1 and C_H2) is the hinge region. It is more flexible and is more exposed to enzymes and chemicals. Thus, papain acts here to produce Fab and Fc fragments.

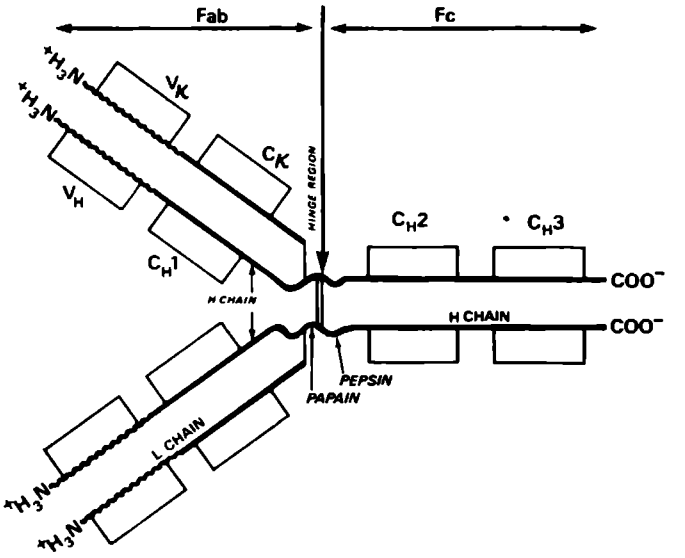

Figure 4–1. A simplified model for an IgG1 (κ) human antibody molecule showing the 4-chain basic structure and domains. V indicates the variable region; C, the constant region; and the vertical arrow, the hinge region. Thick lines represent H and L chains; thin lines represent disulfide bonds.

F(ab)′$_2$ fragment: Digestion of an IgG molecule by the enzyme pepsin produces one F(ab)′$_2$ molecule and small peptides. The F(ab)′$_2$ molecule is composed of 2 Fab units and the hinge region, with intact inter-H chain disulfide bonds, since pepsin cleaves the IgG molecule on the carboxy-terminal side of these bonds.

Disulfide bonds: Chemical disulfide (–S–S–) bonds between cysteine residues are essential for the normal 3-dimensional structure of immunoglobulins. These bonds can be interchain (H chain to H chain, H chain to L chain, L chain to L chain) or intrachain.

Classes: There are 5 classes of immunoglobulins, designated IgG, IgA, IgM, IgD, and IgE (Table 4–1). They are defined by antigenic differences in the C regions of H chains. IgG, IgA, and IgM have been further subdivided into subclasses on the basis of relatively minor antigenic differences in C_H regions.

L chain types: L chains are divided into κ and λ types on the basis of antigenic determinants. Akin to the subclasses of H chains, 4 subtypes of λ chains have been found.

Isotypes: The antigenic differences that characterize the class and subclass of H chains and the type and subtype of L chains. Each normal individual expresses all the isotypes characteristic of the species inasmuch as each isotype occupies a distinctive genetic locus in the genome.

Allotypes: Polymorphic (allelic) forms of H and L chains that exhibit a mendelian pattern of inheritance. The antigenic determinants that characterize allotypes are usually localized to C regions. Thus, a particular isotype may have several alternative (allelic) structures.

Idiotypes: Antigenic determinants that distinguish one V domain from all other V domains.

S value: The S value refers to the sedimentation coefficient of a protein, measured by the technique of Svedberg. S values of normal immunoglobulins range from 6S to 19S (Table 4–1). In general, the larger the S value of a protein, the higher its molecular weight.

Polymers: Immunoglobulins composed of more than a single basic monomeric unit are termed polymers. The main examples are IgA dimers (2 units) and trimers (3 units) and IgM pentamers (5 units).

J chain: This is a polypeptide chain that is normally found in polymeric immunoglobulins.

Secretory component: IgA molecules in secretions are most commonly composed of 2 IgA units, one J chain, and an additional polypeptide, the secretory component.

FOUR-CHAIN BASIC UNIT

Immunoglobulin molecules are composed of equal numbers of heavy and light polypeptide chains, which can be represented by the general formula $(H_2L_2)_n$. The chains are held together by noncovalent forces and usually by covalent interchain disulfide bridges to form a bilaterally symmetric structure (Fig 4–1). It has been shown that all normal immunoglobulins have this basic structure, although some, as we shall see, are composed of more than one 4-chain unit.

Each polypeptide chain is made up of a number of loops or domains of rather constant size (100–110 amino acid residues) formed by the intrachain

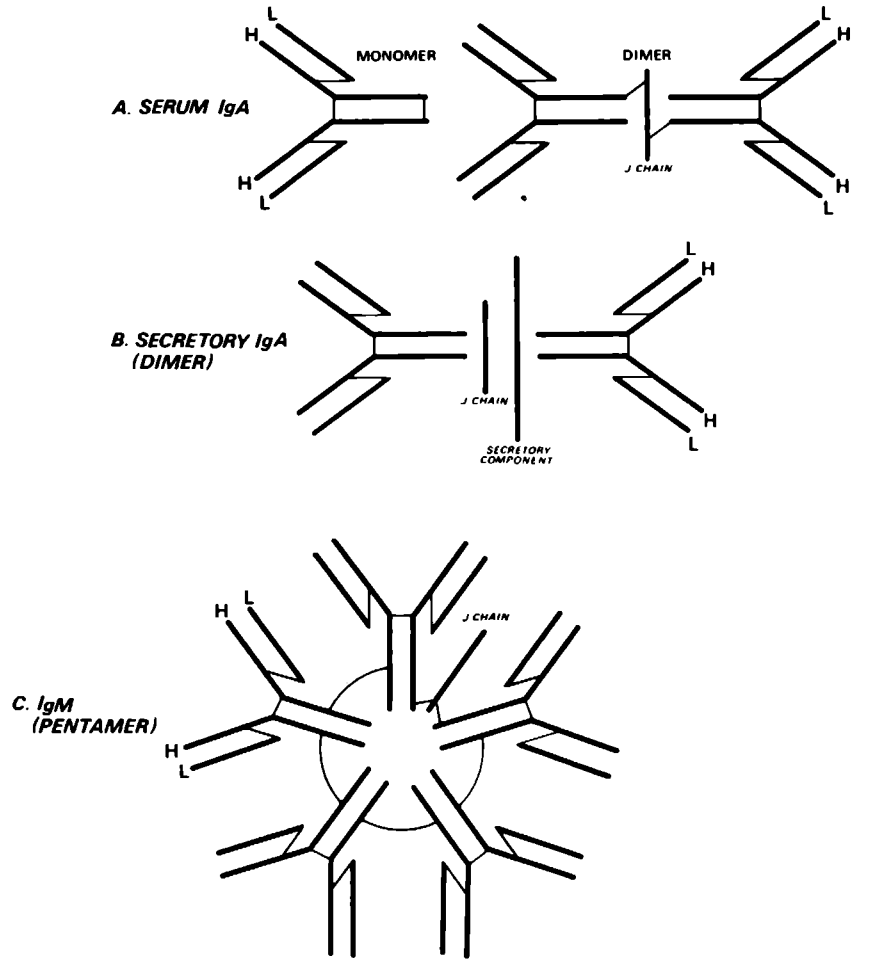

Figure 4–2. Highly schematic illustration of polymeric human immunoglobulins. Polypeptide chains are represented by thick lines; disulfide bonds linking different polypeptide chains are represented by thin lines.

disulfide bonds (Fig 4–1). The N-terminal domain of each chain shows much more variation in amino acid sequence than the others and is designated the variable region to distinguish it from the other relatively constant domains (collectively called the constant region in each chain). The zone where the variable and constant regions join is termed the "switch" region.

Immunoglobulins are rather insensitive to proteolytic digestion but are most easily cleaved about midway in the heavy chain in an area between the first and second constant region domains (C_H1 and C_H2) (Fig 4–1). The enzyme papain splits the molecule on the N-terminal side of the inter-heavy chain disulfide bonds into 3 fragments of similar size: 2 Fab fragments, which include an entire light chain and the V_H and C_H1 domains of a heavy chain; and one Fc fragment, composed of the C-terminal halves of the heavy chains. If pepsin is used, cleavage occurs on the C-terminal side of the inter-H chain disulfide bonds, yielding a large $F(ab)'_2$ fragment composed of about 2 Fab fragments. The Fc fragment is extensively degraded by pepsin. The region in the H chain susceptible to proteolytic attack is more flexible and exposed to the environment than the more compact, globular domains and is known as the "hinge" region. Antigen-binding activity is associated with the Fab fragments or, more specifically, with the V_H and V_L domains, while most of the secondary biologic activities of immunoglobulins (eg, complement fixation) are associated with the Fc fragment.

HETEROGENEITY OF IMMUNOGLOBULINS

As already noted, immunoglobulin molecules comprise a family of proteins with the same basic molecular architecture but which exhibit a vast array

Table 4–1. Properties of human immunoglobulin chains.

Designation	H Chains					L Chains		Secretory Component	J Chain
	γ	α	μ	δ	ε	κ	λ	SC	J
Classes in which chains occur	IgG	IgA	IgM	IgD	IgE	All classes	All classes	IgA	IgA, IgM
Subclasses or subtypes	1,2,3,4	1,2	1,2			. . .	1,2,3,4		
Allotypic variants	Gm(1)–(25)	A2m(1), (2)	. . .	. . .	. . .	Km(1)–(3)†	. . .	. . .	. . .
Molecular weight (approximate)	50,000*	55,000	70,000	62,000	70,000	23,000	23,000	70,000	15,000
V region subgroups	$V_H I$–$V_H IV$					$V_\kappa I$–$V_\kappa IV$	$V_\lambda I$–$V_\lambda VI$		
Carbohydrate (average percentage)	4	10	15	18	18	0	0	16	8
Number of oligosaccharides	1	2 or 3	5	?	5	0	0	?	1

*60,000 for γ3.
†Formerly Inv(1)–(3).

of antigen-binding specificities and different biologic activities. These different activities are, of course, reflections of structural differences dictated by amino acid sequence of the polypeptide chains. This structural heterogeneity has been an obstacle for protein chemists, but plasmacytomas of human and murine origin provide homogeneous (monoclonal) immunoglobulins that have greatly facilitated the study of the amino acid sequence of antibody molecules. Furthermore, it is now possible to produce at will virtually unlimited quantities of monoclonal antibodies of prescribed antigen specificity by somatic cell fusion of plasmacytoma cells with normal antibody-producing cells from immunized animals (see Chapter 17). The monoclonal antibodies produced by such somatic cell hybrids, or "hybridomas," are being used on a vast scale as diagnostic reagents.

Light Chain Types

All L chains have a molecular weight of approximately 23,000 but can be classified into 2 types, kappa (κ) and lambda (λ), on the basis of multiple structural differences in the constant regions which are reflected in antigenic differences (Table 4–1). The 2 types of L chains have been demonstrated in many mammalian species. Indeed, the amino acid sequence homologies between human and mouse κ chains are much greater than those between the κ and λ chains within each species, indicating that the 2 types separated during evolution prior to the divergence of mammalian species.

The proportion of κ to λ chains in immunoglobulin molecules varies from species to species, being about 2:1 in humans. A given immunoglobulin molecule always contains identical κ or λ chains, never a mixture of the 2.

Heavy Chain Classes

Five classes of H chains have been found in humans, based again on structural differences in the constant regions detected by serologic and chemical methods. The different forms of H chain, designated γ, α, μ, δ, and ε (Table 4–1), vary in molecular weight from 50,000 to 70,000, the μ and ε chains possessing 5 domains (one V and four C) rather than the 4 of γ and α chains. The δ chain has an intermediate molecular weight which is believed to be due to an extended hinge region. Likewise, the γ3 chain has an extended hinge region consisting of about 60 amino acid residues, including 14 cysteines, which account for the large number of inter-heavy chain disulfide bonds in IgG3 (Fig 4–3).

The class of the H chain determines the class of the immunoglobulin. Thus, there are 5 classes of immunoglobulins: IgG, IgA, IgM, IgD, and IgE. Two γ chains combined with either two κ or two λ L chains constitute an IgG molecule, the major class of immunoglobulins in serum. Similarly, two μ chains and two L chains form an IgM subunit; IgM molecules are macroglobulins which consist of 5 of these basic 4-chain subunits (Fig 4–2). IgA is polydisperse, comprising 1–5 such units. The other classes (IgD and IgE), like IgG, consist of a single 4-chain unit. The classification and properties of immunoglobulins and their component polypeptide chains are summarized in Tables 4–1 and 4–2.

Subclasses of Polypeptide Chains

Most of the H chain classes have been further subdivided into subclasses on the basis of serologic or physicochemical differences in the constant regions. However, H chains representing the various subclasses within a class are much more closely related to each other than to the other classes. For example, there are 4 subclasses of γ chain in humans, γ1, γ2, γ3, and γ4 (Table 4–2), which yield IgG1, IgG2, IgG3, and IgG4 subclasses of immunoglobulin G molecules, respectively. The C regions of these γ chains are much more homologous to each other than to those of α, μ, δ, or ε chains. In some species, the charge spectra of the IgG subclasses differ sufficiently to permit their isolation by electrophoretic techniques. This is not true in humans, where the subclasses have been recognized by serologic and chemical methods, facilitated by the existence of myeloma proteins.

A noteworthy aspect of the structural differences between the immunoglobulin subclasses is the number

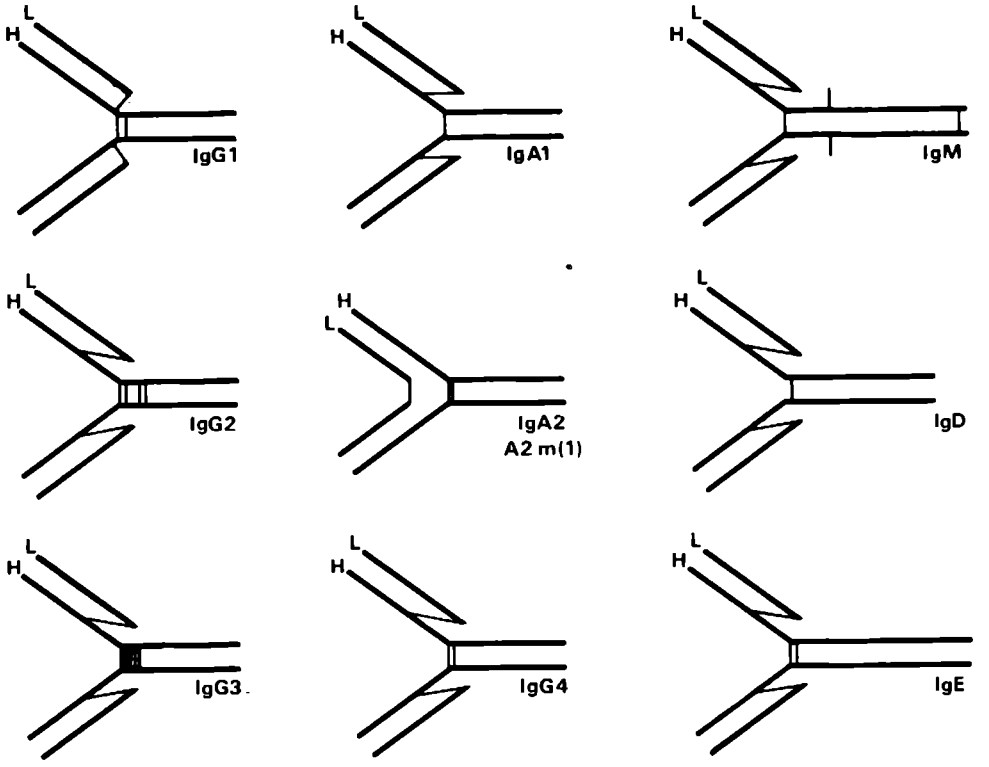

Figure 4–3. Distribution of interchain disulfide bonds in various human immunoglobulin classes and subclasses. H chains are represented by long thick lines and L chains by short thick lines. Disulfide bonds are represented by thin lines. The number of inter-heavy chain disulfide bonds in IgG3 may be as high as 14.

and arrangement of interchain disulfide bridges (Fig 4–3). In IgA2, the L chains are covalently linked to each other instead of to the H chains. In other immunoglobulins, the L–H bond may be formed close to the junction of the V_H and C_H1 domains or, alternatively, near the junction between C_H1 and C_H2 in IgG1.

As for L chains, κ chains do not exhibit C region subclasses, but 4 distinct λ chain forms have been discerned in humans which have apparently arisen by tandem gene duplication. These are called **subtypes** to distinguish them from H chain subclasses which determine the subclass of the intact molecule. Since all H chains may be combined with any of the L chains, the latter play no role in determining the class or subclass of immunoglobulin. Put another way, the complete repertoire of κ and λ chains is found in each immunoglobulin subclass.

Allotypic (Allelic) Forms of Heavy & Light Chains

Some H and L chain isotypes bear genetic markers that are inherited in typical mendelian fashion. These **alternative forms at a given genetic locus are called allotypes.** In humans, allelic forms have been found for γ and α H chains and κ L chains. The allotypes associated with γ chains are designated "Gm" (for gamma), those associated with α chains are termed "Am," and those associated with κ L chains are called "Inv" (abbreviation of a patient's name). Thus far, allotypic forms of λ L chains or the H chains of IgM, IgD, and IgE have not been found.

Allotypy has been detected using homologous (same species) antisera that react with antigenic determinants foreign to the immunoglobulins of the host. For example, mothers may become immunized to paternal allotypic determinants on fetal immunoglobulins during the course of pregnancy. Alternatively, immunization may result from blood transfusions. Another source of detecting reagents has been the sera of some patients with rheumatoid arthritis, which contain "rheumatoid factors" reactive with IgG from some (not all) normal individuals (see Chapter 21). Such rheumatoid factors detect allotypic determinants. The structural differences that account for allotypic determinants usually involve only one or, at most, several amino acid substitutions in the constant regions of H and L chains.

Table 4–2. Properties of human immunoglobulins.

	IgG	IgA	IgM	IgD	IgE
H chain class	γ	α	μ	δ	ϵ
H chain subclass	$\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$	$\alpha 1$, $\alpha 2$	$\mu 1$, $\mu 2$		
L chain type	κ and λ	κ and λ	κ and λ	κ and λ	κ and λ
Molecular formula	γ_2L_2	α_2L_2* or $(\alpha_2L_2)_2$SC†J‡	$(\alpha_2L_2)_5$J‡	δ_2L_2	ϵ_2L_2
Sedimentation coefficient (S)	6–7	7	19	7–8	8
Molecular weight (approximate)	150,000	160,000* 400,000§	900,000	180,000	190,000
Electrophoretic mobility (average)	γ	Fast γ to β	Fast γ to β	Fast γ	Fast γ
Complement fixation (classic)	+	0	++++	0	0
Serum concentration (approximate; mg/dL)	1000	200	120	3	0.05
Serum half-life (days)	23	6	5	2–8	1–5
Placental transfer	+	0	0	0	0
Reaginic activity	?	0	0	0	++++
Antibacterial lysis	+	+	+++	?	?
Antiviral activity	+	+++	+	?	?

*For monomeric serum IgA. ‡J chain.
†Secretory component. §For secretory IgA.

SECRETORY COMPONENT & J CHAIN

Immunoglobulins are present not only in serum but also in various body secretions such as saliva, nasal secretions, sweat, breast milk, and colostrum. IgA is the predominant immunoglobulin class in the external secretions of most species. IgA usually exists in human serum as a 4-chain unit of approximately molecular weight 160,000 (7S). This unit may polymerize to give disulfide-bonded polymers with 8-chain, 12-chain, or larger structures. The IgA in secretions consists of two 4-chain units associated with one of each of 2 additional chain types, the secretory component and the J chain (Tables 4–1 and 4–2). The secretory component is associated only with IgA and is found almost exclusively in body secretions. The J chain is associated with all polymeric forms of immunoglobulins that contain 2 or more basic units. Fig 4–2 shows simplified models of secretory IgA and various polymeric serum immunoglobulins. Evidence suggests that binding of an IgA to secretory component or J chain (or both) may promote the polymerization of additional monomeric 4-chain basic units. The secretory component may exist in free form or bound to IgA molecules by strong noncovalent interactions. The binding does not usually involve covalent bonding, although disulfide bonds have been implicated in a small fraction of human secretory IgA molecules. The secretory component is synthesized by nonmotile epithelial cells near the mucous membrane where secretion occurs. Its function may be to enable IgA antibodies to be transported across mucosal tissues into secretions.

The secretory component is a single polypeptide chain of approximately molecular weight 70,000. The carbohydrate content is high but not precisely known (Table 4–1). Its amino acid composition differs appreciably from that of every other immunoglobulin polypeptide chain, including J chain. No close structural relationship exists between the secretory component and any immunoglobulin polypeptide chain. Indeed, secretory component can be found free in secretions of individuals who lack measurable IgA in their serum or secretions. The secretory component has an electrophoretic mobility in the fast β range and shows little tendency to form aggregates in phosphate-buffered saline at pH 7.3.

The J chain is a small glycopeptide with an unusually high content of aspartic acid and glutamic acid. The J chain has a fast electrophoretic mobility on alkaline gels owing to its highly acidic nature. Equilibrium centrifugation in 5 molar guanidine hydrochloride indicates that the J chain has a molecular weight of approximately 15,000. Physicochemical studies indicate that the J chain molecule is very elongated, with an axial ratio of approximately 18.

Quantitative measurements indicate that there is a single J chain in each IgM pentamer or polymeric IgA molecule. The J chain is covalently bonded to the penultimate cysteine residue of α and μ chains. Whether or not the J chain is required for the proper polymerization of the IgA and IgM basic unit is controversial. Polymeric immunoglobulins of certain lower vertebrates such as nurse shark and paddlefish are apparently devoid of J chain. These observations indicate that J chain is not an absolute requirement for polymerization of the immunoglobulin basic units. Nevertheless, the presence of J chain does facilitate the polymerization of basic units of IgA and IgM molecules into their appropriate polymeric forms.

CARBOHYDRATE MOIETIES OF IMMUNOGLOBULINS

Significant amounts of carbohydrate are present in all immunoglobulins in the form of simple or complex

side chains covalently bonded to amino acids in the polypeptide chains (Table 4–1).

The function of the carbohydrate moieties is poorly understood. They may play important roles in the secretion of immunoglobulins by plasma cells and in the biologic functions associated with the C regions of H chains.

The attachment in most cases is by means of an N-glycosidic linkage between an N-acetylglucosamine residue of the carbohydrate side chain and an asparagine residue of the polypeptide chain. However, other linkages have also been observed, including an O-glycosidic linkage between an amino sugar of an oligosaccharide side chain and a serine residue of the polypeptide chain. In general, carbohydrate is found in only the secretory component, the J chain, and the C regions of H chains; it is not found in L chains or the V regions of H chains. Exceptions to this rule have been found in a small number of myeloma proteins. The secretory component has more carbohydrate than either the α chain or the L chain, which accounts for the higher carbohydrate content in secretory IgA than in serum IgA. Studies on monoclonal immunoglobulins indicated that IgM and IgE generally have an average of 5 oligosaccharides each; IgG, one; and IgA, 2 or 3 oligosaccharides. This agrees with the overall carbohydrate content of immunoglobulins, since IgM, IgD, and IgE have the largest amounts of carbohydrate, followed by IgA and then by IgG (Table 4–1). However, these studies were performed on a limited number of monotypic immunoglobulins. In view of the findings that (1) different myeloma proteins of the same class or subclass may differ from one another in carbohydrate content, (2) an individual myeloma protein occasionally exhibits microheterogeneity with respect to its carbohydrate content, and (3) V regions of a small number of immunoglobulin polypeptide chains contain carbohydrate, it is incorrect to assume that all immunoglobulins belonging to a given class or subclass have the same number of oligosaccharide side chains.

BIOLOGIC ACTIVITIES OF IMMUNOGLOBULIN MOLECULES

As we have already noted, immunoglobulins are bifunctional molecules which bind antigens and, in addition, initiate other biologic phenomena which are independent of antibody specificity. These 2 kinds of activity can each be localized to a particular part of the molecule: antigen binding to the combined action of the V regions of H and L chains, and the other activities to the C regions of H chains. These latter activities, some of which are listed in Table 4–2, will be considered in this section.

Immunoglobulin G (IgG)

In normal human adults, IgG constitutes approximately 75% of the total serum immunoglobulins. Within the IgG class, the relative concentrations of the 4 subclasses are approximately as follows: IgG1, 60–70%; IgG2, 14–20%; IgG3, 4–8%; and IgG4, 2–6%. These figures vary somewhat from individual to individual and correlate weakly with the presence of certain H chain C region allotypic markers (see Chapter 3). Thus, the capacity of a given individual to produce antibodies of one or another IgG subclass may be under genetic control.

IgG is the only class of immunoglobulin that can cross the placenta in humans, and it is responsible for protection of the newborn during the first months of life (see Chapter 34). The subclasses are not equally endowed with this property, IgG2 being transferred more slowly than the others. The adaptive or biologic value of this inequality, if any, is obscure.

IgG is also capable of fixing serum complement (see Chapter 10), and once again the subclasses function with unequal facility in the following order: IgG3 > IgG1 > IgG2 > IgG4. IgG4 is completely unable to fix complement by the classic pathway (binding of C1q) but may be active in the alternative pathway. The specific location of the C1q binding site on the IgG molecule appears to reside in the CH2 domain.

Macrophages bear surface receptors that bind IgG1 and IgG3 and their Fc fragments. The passive binding of antibodies by such Fc receptors is responsible for "arming" macrophages, which can then function in a cytotoxic fashion (see Chapter 9). The specific location of the Fc receptor binding site on IgG1 and IgG3 molecules seems to be in the CH3 domain.

Immunoglobulin A (IgA)

IgA is the predominant immunoglobulin class in body secretions (see Chapter 12). Each secretory IgA molecule consists of two 4-chain basic units and one molecule each of secretory component and J chain (Fig 4–2). The molecular weight of secretory IgA is approximately 400,000. Secretory IgA provides the primary defense mechanism against some local infections owing to its abundance in saliva, tears, bronchial secretions, the nasal mucosa, prostatic fluid, vaginal secretions, and mucous secretions of the small intestine. The predominance of secretory IgA in membrane secretions led to speculation that its principal function may not be to destroy antigen (eg, foreign microbial organisms or cells) but rather to prevent access of these foreign substances to the general immunologic system. IgA normally exists in serum in both monomeric and polymeric forms, constituting approximately 15% of the total serum immunoglobulins.

Immunoglobulin M (IgM)

IgM constitutes approximately 10% of normal immunoglobulins and normally exists as a pentamer with a molecular weight of about 900,000 (19S). IgM antibody is prominent in early immune responses to most antigens and predominates in certain antibody responses such as "natural" blood group antibodies. IgM (with IgD) is the major immunoglobulin expressed on

the surface of B cells. IgM is also the most efficient complement-fixing immunoglobulin, a single molecule bound to antigen sufficing to initiate the complement cascade (see Chapter 10).

Immunoglobulin D (IgD)

The IgD molecule is a monomer, and its molecular weight of approximately 180,000 (7–8S) is slightly higher than that of IgG. This immunoglobulin is normally present in serum in trace amounts (0.2% of total serum immunoglobulins). It is relatively labile to degradation by heat and proteolytic enzymes. There are isolated reports of IgD with antibody activity toward certain antigens, including insulin, penicillin, milk proteins, diphtheria toxoid, nuclear antigens, and thyroid antigens. However, the main function of IgD has not yet been determined. IgD (with IgM) is the predominant immunoglobulin on the surface of human B lymphocytes, and it has been suggested that IgD may be involved in the differentiation of these cells.

Immunoglobulin E (IgE)

The identification of IgE antibodies as reagins and the characterization of this immunoglobulin class marked a major breakthrough in the study of the mechanisms involved in allergic diseases (see Chapters 15 and 24). IgE has a molecular weight of approximately 190,000 (8S). It constitutes only 0.004% of the total serum immunoglobulins but binds with very high affinity to mast cells via a site in the Fc region. Upon combination with certain specific antigens called allergens, IgE antibodies trigger the release from mast cells of pharmacologic mediators responsible for the characteristic wheal-and-flare skin reactions evoked by the exposure of the skin of allergic individuals to allergens. IgE antibodies provide a striking example of the bifunctional nature of antibody molecules. "Allergen" is an alternative term used by allergists for any antigen that stimulates IgE production. IgE antibodies bind allergens through the Fab portion, but the binding of IgE antibodies to tissue cells is a function of the Fc portion. Like IgG and IgD, IgE normally exists only in monomeric form.

THE VARIABLE REGION

The V regions, comprising the N-terminal 110 amino acids of the L and H chains, are quite heterogeneous. Indeed, no 2 human myeloma chains from different patients have been found to have identical sequences in the V region. However, distinct patterns are discernible, and V regions have been divided into 3 main groups based on degree of amino acid sequence homology. These are the V_H group for H chains, V_κ group for κ L chains, and V_λ group for λ L chains. These V region groups are only associated with the appropriate C region subclasses or subtypes for that particular polypeptide. For example, a V_H sequence will only be found on an H chain, never on a κ or λ light chain, and so forth. However, a particular V_H sequence may associate with any C_H class (γ, α, μ, δ, or ϵ). The genes coding for associated V and C regions are probably linked (see Chapter 5).

V Region Subgroups

When the sequences of the V regions of κ chains are compared, they can be further divided into 4 subgroups which have substantial homologies. The subgroups differ from one another principally in the length and position of amino acid insertions and deletions and bear much closer structural homology to each other than to λ or H chain V regions. Similar subdivisions have been made in H chain V regions and λ chain V regions.

Hypervariable Regions

The V regions are not uniformly variable across their spans but consist of relatively invariant positions, which define the type and subgroup to which the V region belongs, as well as highly variable zones or "hot spots." A plot of the known variations versus position in the sequence reveals 3 or 4 peaks, depending on the chain type. These peaks of extreme variability are known as **hypervariable regions** and have been shown to be intimately involved in the formation of the antigen binding site. L chains appear to have 3 hypervariable regions, while H chains have 4, although only 3 of the 4 have been shown to contribute to the antigen-binding site (Fig 4–4). The approximate locations of the hypervariable regions in each chain are shown in Fig 4–5.

Idiotypes

The term idiotype denotes the unique V region sequences produced by each clone of antibody-forming cells. Idiotypic antigenic determinants of immunoglobulin molecules were identified by immunizing animals with specific antibodies raised against a particular antigen in genetically similar animals. The only antigenic differences between the immunoglobulins of the donor and recipient were the unique V region sequences related to the specificity of the antibody. Thus, responses were restricted to such determinants.

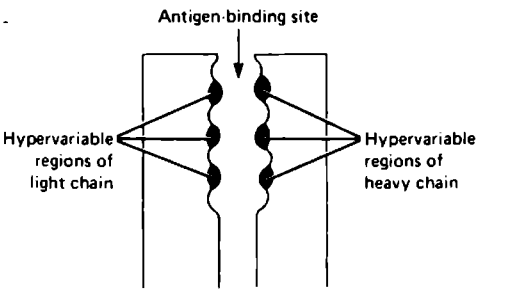

Figure 4–4. Schematic depiction of how the hypervariable regions in each heavy and light chain might form an antigen-binding site of an antibody molecule.

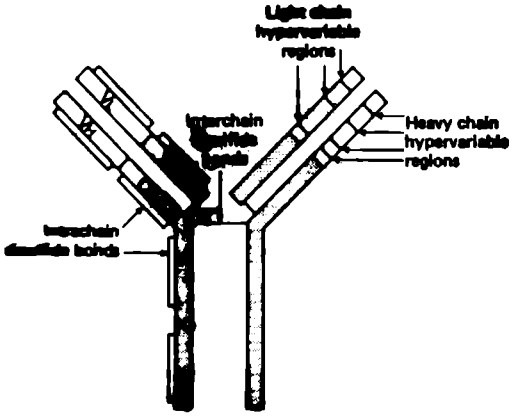

Figure 4–5. Schematic model of an IgG molecule showing approximate positions of the hypervariable regions in heavy and light chains.

It is also possible to immunize across species lines to obtain anti-idiotype antisera, but in this case the antisera must be carefully absorbed with immunoglobulins from the donor species to render them specific for idiotypic markers.

In some cases, the reaction between anti-hapten antibody and anti-idiotypic antisera raised against that anti-hapten antibody can be inhibited by the hapten, indicating that the idiotypic antigenic determinants are close to or within the antigen-binding site of the antibody molecule. An antibody to idiotypic determinants is therefore regarded as an immunologic marker for the antibody combining site. Although it is not yet formally proved, idiotypic determinants are believed to be associated with hypervariable regions which determine antibody specificity.

It seems legitimate to extend the term idiotype to any combination of a particular L chain V region with a particular H chain V region. That is, any such combination will express a unique idiotypic specificity. Since any L chain may combine with any H chain and a common pool of VH regions is shared by the 5 different classes of H chains, it follows that idiotypic determinants may be shared by different immunoglobulin classes. Idiotypic determinants are heritable, at least in some cases, as observed in certain inbred strains of mice.

THE THREE-DIMENSIONAL STRUCTURE OF IMMUNOGLOBULINS

Although the inference that the polypeptide chains of immunoglobulin molecules are folded into compact globular domains separated by short linear stretches was derived initially from amino acid sequence studies, confirmation of this structural model required examination of crystallized immunoglobulins or their component parts by x-ray diffraction analysis. This work has shown that all domains have a characteristic pattern of folding, regardless of their origin. Thus, V region and C region domains from L chains and H chains all have a very similar appearance. In addition, there is close physical approximation between corresponding domains, ie, V_H and V_L, C_H1 and C_L, and the identical H chain domains in the Fc portion. X-ray diffraction analysis of a crystallized myeloma protein complexed with hapten (see Chapter 3) revealed that the contact points between antigen and the antibody combining site are located in the hypervariable regions of the H and L chains.

Other evidence in favor of the domain model has come from limited proteolysis of immunoglobulins, in which the major products appear to consist of one or more domains (as expected, based on the model, since the areas between the domains are more exposed and consequently more susceptible to enzymatic attack). It has also been found that some of the proteins present in patients with H chain disease (see Chapter 22) have large deletions involving the entire C_H1 domain.

All domains, including those from the same polypeptide chain, different polypeptide chains, the same molecules, and different molecules, show a significant degree of amino acid homology. This led to the hypothesis that all immunoglobulin polypeptide chains evolved by a process of tandem gene duplication from a common ancestor that was equivalent to one domain.

CELL SURFACE IMMUNOGLOBULINS

Although, as noted earlier, IgM and IgD constitute the predominant membrane immunoglobulins, all classes of immunoglobulins have been found on the surfaces of B lymphocytes, where they function as antigen receptors. The membrane and secreted forms of μ, δ, and γ chains (and presumably α and ϵ chains as well) differ in structure. The membrane forms have an additional carboxy-terminal sequence of approximately 40 amino acid residues, which begins with a highly acidic sequence of 12–14 residues and terminates with a strikingly hydrophobic sequence of about 26 residues. The hydrophobic portion of the segment is believed to represent the transmembrane component anchoring the heavy chain in the cell membrane. It is similar in hydrophobicity and length to known transmembrane segments of other proteins, and it satisfies the requirements for the formation of a membrane-spanning alpha helix.

Whereas the acidic part of the membrane segment shows little amino acid sequence homology between heavy chain classes, the hydrophobic sequences of μ and γ chains show substantially greater homology than do the constant region domains of those classes. This sequence conservation is puzzling, because transmembrane segments of other proteins seem to have little in common besides length and hydrophobicity.

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Immunoglobulins II: Gene Organization & Assembly

5

Stanley J. Korsmeyer, MD, & Thomas A. Waldmann, MD

RECOMBINATIONAL GERM LINE THEORY

The process by which an individual can generate approximately 10^6–10^8 different antibody specificities has been clarified to a great extent in recent years. The first available amino acid sequence data from myeloma proteins revealed that these homogeneous immunoglobulins, composed of light and heavy polypeptide chains, varied markedly in their amino-terminal portion (variable regions) but had nearly invariant sequences in their carboxy-terminal portions (constant regions). The fact that the amino-terminal portions of both the heavy and the 2 light chain immunoglobulin classes (kappa and lambda) appeared to duplicate and diverge over evolutionary time while their carboxy-terminal portions remained unchanged posed a molecular genetic dilemma. How could genes coding for these unusual polypeptides undergo numerous changes in part of their sequences while faithfully conserving another portion? This apparent dichotomy prompted Dreyer and Bennett in 1965 to propose that 2 genes would code for a single immunoglobulin polypeptide chain! They further speculated that multiple different variable region genes would exist separated from a single constant region gene and that these segments would be joined together at the DNA level. Such an elegant recombinational model would allow for recognition of a vast array of antigens by the multiple variable regions; yet it would also ensure that the invariant functions provided by the constant regions would be conserved.

DISCONTINUOUS IMMUNOGLOBULIN GENES

Within recent years, investigators using recombinant DNA technology have confirmed the **recombinational germ line theory** Dreyer and Bennett had proposed. Direct analysis of immunoglobulin genes by Tonegawa and Leder as well as others revealed that the variable (V) and constant (C) region portions of immunoglobulin were indeed separately encoded and located on different fragments of DNA. However, the variable portion of light chain immunoglobulin proved to be encoded by 2 separate gene segments, not one.

For example, the kappa light chain's initial gene segment, the variable (V_κ) region gene, is foreshortened, coding for only the first 95 amino acids of the variable region protein (Fig 5–1). The remaining 13 amino acids (positions 96–108) of the variable portion of the molecule are contributed by one of 5 alternative segments termed joining (J_κ) segments. As shown in Fig 5–1, there is but a single constant kappa (C_κ) region gene carried on yet a third segment. The C_κ and J_κ regions are separated within DNA by a long stretch of intervening sequence (IVS). Shorter intervening sequences separate each of the joining (J_κ) segments from each other. Such intervening sequences themselves do not for the most part code for any recognizable protein or function. Many other eukaryotic structural genes are also discontinuous in their organization, being coded for by pieces of structural gene information separated by intervening sequences. These intervening sequences may play a crucial role in determining the evolutionary integrity of structural genes or even in modifying their expression.

SOMATIC ASSEMBLY OF IMMUNOGLOBULIN GENE SEGMENTS CREATES A FUNCTIONAL ANTIBODY GENE

At some point during the differentiation of a pluripotential stem cell into a terminally differentiated kappa-producing plasma cell, a process of DNA rearrangement must occur. This recombination of DNA joins one of many germ line variable (V_κ) regions with a particular joining (J_κ) region (Fig 5–1). This rearranged allele is transcribed, and the remaining intervening sequences are removed at a step known as RNA splicing. The final mature mRNA is translated into the complete light chain product. The mRNA, in addition, contains the 19 codons that encode the short hydrophobic leader peptide responsible for the transmembrane passage of this polypeptide. Each germ line V_κ region has its own leader sequence (L) separated from the V_κ region by a short intervening sequence. The leader sequence region (L) codes for positions -19 to -4 and is joined to the main V_κ region -3 to 95 by RNA splicing. The leader peptide is present on the cytoplasmic kappa chain but is cleaved off during secretion.

Nucleic acid sequence determinations through recombined and germ line variable (V_κ) and joining (J_κ)

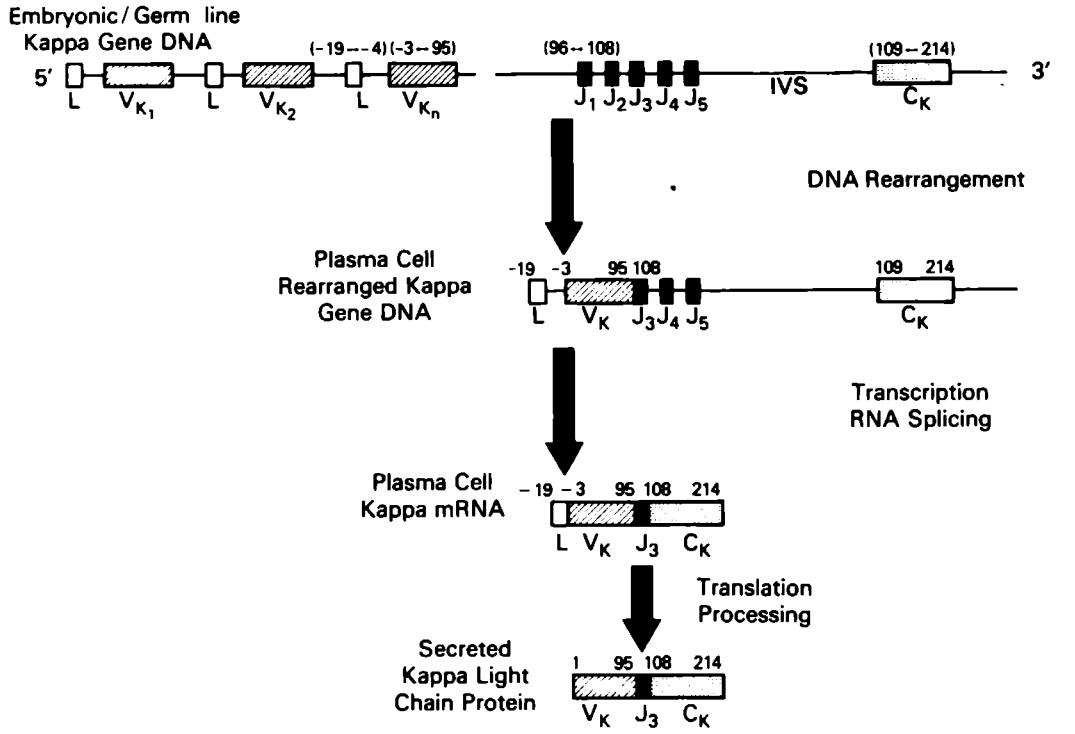

Figure 5–1. Schematic representation of the human kappa gene locus. Multiple germ line variable (V_κ) regions exist, each accompanied by a leader (L) sequence. There are 5 alternative joining (J_κ) segments, each coding for amino acid positions 96–108. There is only one constant (C_κ) region per allele. DNA rearrangement joins a single V_κ and J_κ segment. The remaining intervening sequences (IVS) are removed by RNA splicing.

regions established that the codon for the 96th amino acid was the site at which these 2 fragments usually join. Furthermore, a specific set of nucleic acid bases that flank these germ line variable (V) and joining (J) segments appears to mediate V/J joining. At the immediate 3′ side of each germ line variable (V_κ) region and the immediate 5′ side of each germ line joining (J_κ) region is a heptanucleotide, which is an inverted repeat, or so-called palindrome, of CAC $\begin{matrix}A\\T\end{matrix}$ GTG (Fig 5–2). Following a spacer of 11 or 12 nucleotides on the 3′ side of each V_κ gene heptanucleotide is an A,C-rich nonanucleotide. This corresponds to a complementary G,T-rich nonanucleotide that is separated by 22 or 23 nucleotides from its heptanucleotide on the 5′ side of each germ line joining (J_κ) segment. The length of the spacers between the heptanucleotides and nonanucleotides (either 11 or 22 base pairs) is curiously equal to either one or 2 turns of the DNA helix. In both light chain classes, kappa and lambda, it appears that gene segments with 11 base pair spacers always pair with segments having 22 base pair spacers. The fact that both the 7-base-pair palindrome and the 9-base-pair areas of homology have been remarkably conserved throughout evolution strongly suggests that they are active participants in bringing a V and J region together. One possibility is that the 2 heptanucleotides and the 2 nonanucleotides would base pair, creating a stemlike structure that would facilitate recombination between the strands (Fig 5–2). Any flanking DNA located between the juxtaposed V_κ and J_κ segments appears to be deleted from the genome during this process of DNA rearrangement. The precise process of recombination will undoubtedly prove to be even more detailed than shown in Fig 5–2. Current data suggest that a complex process of DNA inversion may actually flip the orientation of V and J segments before deletion and rejoining of DNA occur.

The actual nucleotide base within the triplicate codon at which a variable (V) and joining (J) region align can vary and thus generate additional amino acids at this 96th position. It is noteworthy that the 96th amino acid resides within one of the 3 subdivisions of the variable light chain known to have the highest rates of amino acid differences. This subdivision of the variable light chain, the third hypervariable region, is also intimately involved in determining an antibody's antigenic specificity. Therefore, the flexible frame of recombination within this 96th codon may well create amino acid substitutions that ultimately generate further antibody diversity.

Thus, this is an elaborate system which utilizes movable gene segments and exploits a flexible frame of recombination at the point of joining to maximize

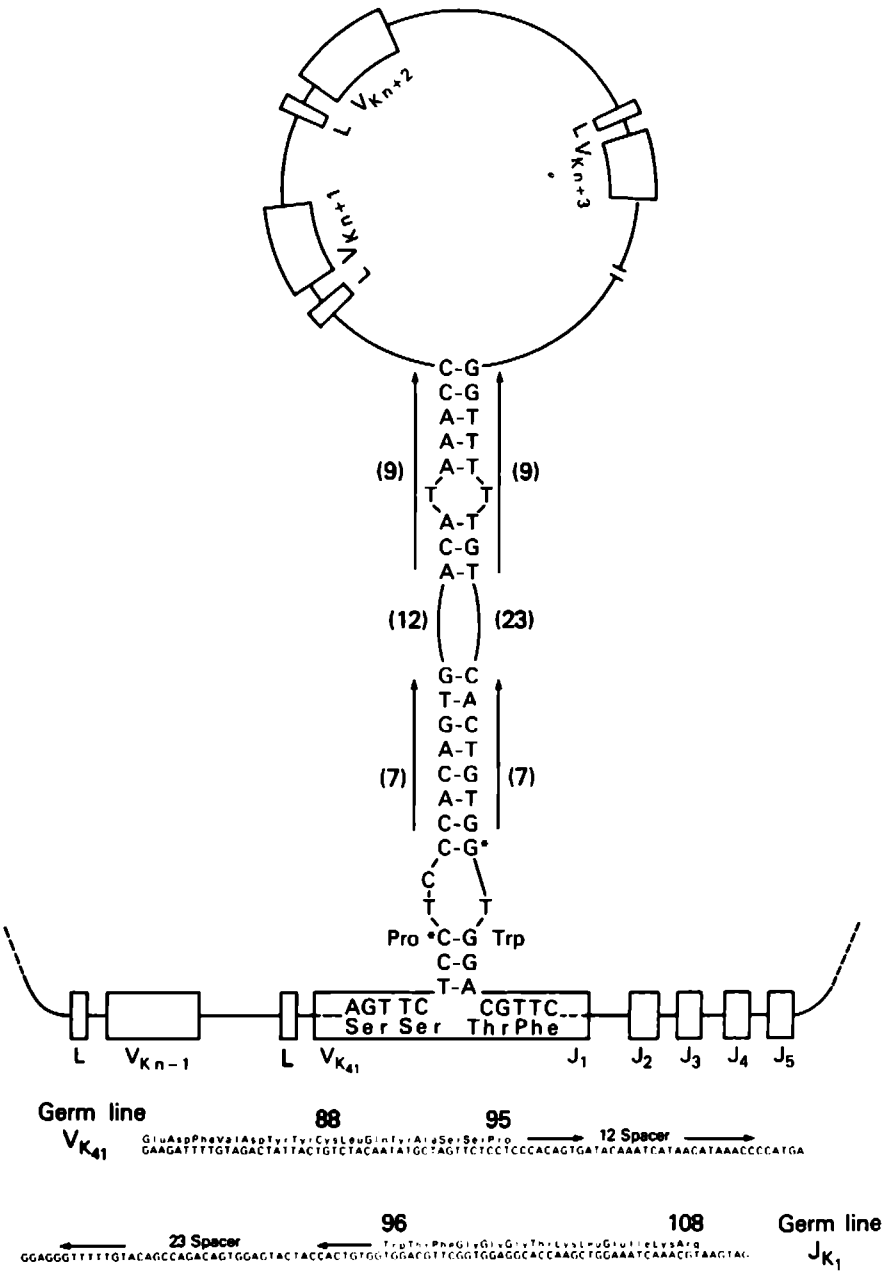

Figure 5–2. Hypothetical recombinational model for V_κ/J_κ joining. A stem structure may form between the palindromic heptanucleotide (CAC $\frac{A}{T}$ GTG) and homologous nonanucleotides (solid arrows) located to the 3′ side of a particular germ line variable region (V_κ41) and those that occur in reverse order on the 5′ side of a germ line joining segment (J_κ1). Spacers of 12 or 23 nucleotides separate the heptanucleotides and nonanucleotides (solid arrows) of the V_κ and J_κ regions, respectively. An asterisk (*) denotes the specific bases at which this particular V_κ and J_κ region are joined together. Any DNA located between the recombining V_κ and J_κ region is apparently deleted.

the antibody diversity that can be generated from limited germ line information. However, such attempts at gene recombination are frequently imprecise, failing to properly align these segments. Such aberrant or abortive rearrangements are mistaken events incapable of forming a complete light chain polypeptide. All kappa-producing B cells or plasma cells must, of course, contain one effective V/J recombination corresponding to the kappa chain that is produced. It is known that each B cell expresses only one (either the maternal or paternal copy) of its 2 inherited kappa alleles. B cells are thus said to display the phenomenon of allelic exclusion. The nonexpressed or excluded allele within B cells has been found to be either in the germ line position or, quite frequently, to be aberrantly rearranged or even deleted. Thus, several different gene patterns may prevent expression of the other allele and perhaps account for the phenomenon of allelic exclusion.

LIGHT CHAIN GENE ORGANIZATION

The kappa light chain makes up 95% of mouse and two-thirds of human light chain protein. At the DNA level, this gene complex has been highly conserved during the 70 million years the 2 species have been divergent. The general design of the human kappa gene complex diagrammed in Fig 5–1 reveals this to be the simplest functional gene system of all the immunoglobulin classes. Multiple germ line variable (V_κ) regions exist within the genome as sets or families of genes, perhaps corresponding to the subgroups of variable regions as defined by the amino acid sequences of kappa chains. The exact number of V_κ gene regions available in the germ line repertoire is unknown, but estimates of several hundred have been made. Five functional joining (J_κ) segments exist for humans, compared to 4 functional plus one nonfunctional segment in the mouse. There is but a single constant kappa (C_κ) region found on chromosome number 2 in humans on the short arm at band 2p11, whereas the κ genes are on chromosome number 6 in the mouse.

Humans utilize lambda light chain genes in one-third of their immunoglobulins, whereas the mouse uses lambda less than 5% of the time. On the basis of somatic cell genetics and in situ chromosomal hybridization studies, the lambda genes have been assigned to the 16th mouse chromosome and the 22nd human chromosome on the long arm at band 22q11. The variable lambda gene (V_λ) region repertoire is markedly contracted in the mouse, there being perhaps only 2 such regions present, whereas the human V_λ gene repertoire is much larger. In distinct contrast to the kappa gene system, there are multiple duplicated constant (C_λ) regions arranged in tandem along chromosome 22 in humans. The first 3 constant regions in this locus correspond to the distinct nonallelic C_λ regions bearing the amino acid markers of Mcg, Ke^-Oz^-, and Ke^-Oz^+ (Fig 5–3) that were identified by serologic and amino acid analysis of human lambda light chain proteins. The Ke (Kern) and Oz markers actually represent amino acid differences identified on different lambda Bence Jones proteins. The Mcg subclass of lambda contains only an additional 3 amino acid changes. Thus, the lack of substantial differences among these separate C_λ regions may reflect a relatively recent duplication of these genes. This human lambda gene locus has proved to be rather varied in the normal population, as evidenced by what is referred to as a restriction fragment length polymorphism (Fig 5–3). A DNA restriction fragment is produced by a restriction endonuclease, which is an enzyme that recognizes a specific base pair sequence and reproducibly

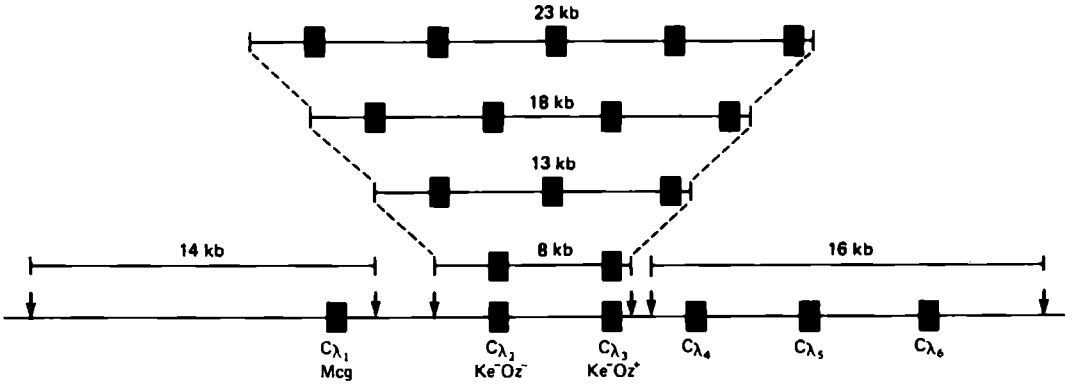

Figure 5–3. Schematic representation of the polymorphic germ line human lambda constant (C_λ) regions. In its most contracted form, six C_λ regions are found on EcoRI-generated restriction endonuclease fragments of 14 kb, 8 kb, and 16 kb. Multiple allelic forms of the central EcoRI fragment exist and are either 8, 13, 18, or 23 kb in size. The incremental enlargement of this center fragment increases the number of C_λ regions present. Mcg, Ke, and Oz represent amino acid markers that distinguish the separate C_λ regions.

cuts DNA only where this site appears. The genetic polymorphism in the lambda locus is manifested as a variation in the length of the DNA fragment that composes the central portion of this locus when cut with the restriction endonuclease, EcoRI. In its simplest form, this central EcoRI fragment is but 8 kilobase (kb) pairs in size and contains the two C_λ regions, Ke^-Oz^- and Ke^-Oz^+ (Fig 5–3). As is evident in Fig 5–3, multiple allelic forms of this center fragment exist and can be either 8 kb, 13 kb, 18 kb, or 23 kb in size. The incremental enlargement of this central EcoRI fragment is a series of 5-kb additions. In fact, this internally duplicated 5-kb fragment itself actually contains a single C_λ region. Therefore, while the 8-kb allele has only 2 internal C_λ regions, the 23-kb alternative possesses five C_λ regions on this central fragment. This variable amplification of the C_λ genes in this locus means that the actual number of C_λ genes in humans can vary between 6 and 9 on this portion of chromosome 22 depending upon whether the 8-, 13-, 18-, or 23-kb allele is inherited. Since a separate J_λ region may be associated with each of these C_λ regions, such gene duplications may actually generate an increase in the final antibody diversity. In addition, this EcoRI restriction endonuclease-defined variation in DNA fragment length (restriction fragment length polymorphism) also serves as an allelic marker located directly at the DNA level of chromosome 22. Such variation in the location of restriction endonuclease sites in different individuals provides a useful genetic marker to facilitate the mapping of inherited diseases to specific chromosomes in humans.

HEAVY CHAIN GENE ASSEMBLY

The general scheme of heavy chain gene organization, while similar to that of light chain genes, is somewhat more complex (Fig 5–4). This additional complexity enables heavy chains to make an even greater contribution to the generation of an individual's total antibody diversity. Three (not 2) segments of DNA must be joined to assemble a gene coding for the entire variable portion of the heavy chain. When nucleic acid sequence determinations of germ line variable heavy (V_H) and joining heavy (J_H) gene regions were obtained, it was apparent that they could not account for all of the amino acid positions found in the variable heavy region proteins produced by B cell hybridomas or plasmacytomas. In addition, the spacing between the heptanucleotide and nonanucleotide recombination sequences that flank these gene segments was 22

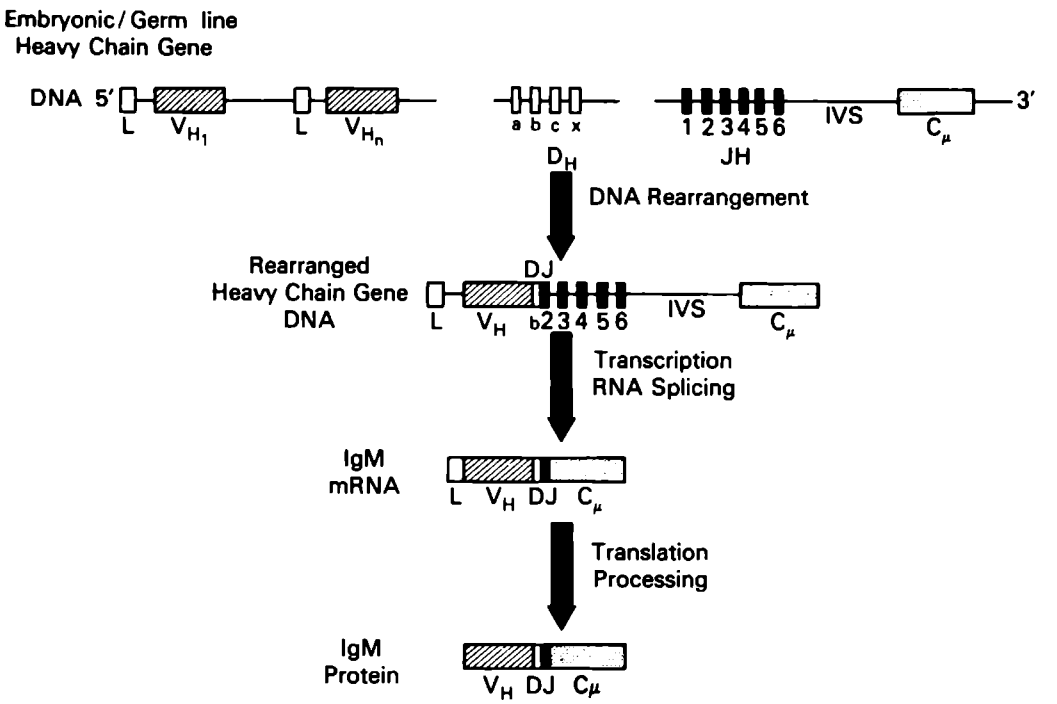

Figure 5–4. Schematic model of the organization and assembly of the human heavy chain gene. In addition to multiple variable (V_H) regions with leader (L) sequences, there are 6 functional joining (J_H) segments and families of diversity (D_H) segments. Single V_H, D_H, and J_H regions are recombined at the DNA level. RNA splicing later removes the residual intervening sequences (IVS).

base pairs for both the V_H and J_H gene regions. This violated the association discussed above of an 11 base pair with a 22-base-pair spacer found in all V/J recombinations of light chain genes. This constellation of findings suggested the existence of an additional set of germ line gene segments designated the diversity (D_H) gene region. Sets or families of such predicted germ line diversity (D_H) gene segments have been demonstrated within the genome. These germ line D_H segments are flanked on each side by the same heptanucleotide and nonanucleotide recombination sequences previously discussed. These 2 sequences are themselves separated by the predicted 11-base-pair spacers. These germ line D_H segments frequently have more than one open reading frame, and several amino acid sequences can thus be produced by a single gene sequence. The D_H segment accounts for a sizable portion of the heavy chain's third hypervariable or complementarity-determining region (CDR_3), which is an integral component of an antibody's specificity and frequently its idiotype. As in the V/J joining of light chain genes, these 3 separate germ line gene segments ($V_H/D_H/J_H$) of heavy chains can once again utilize several frames of recombination when joining together to generate the variable portion of the heavy chain and thus generate even further diversity. Moreover, unexpected nucleotides found at the sites of V_H/D_H and D_H/J_H juncture may reflect exonuclease-mediated loss of base pairs followed by insertion of new nucleotides. These inserted bases are referred to as N regions, are rich in guanosine and cytosine, and may result from the action of terminal deoxynucleotidyl transferase (TdT).

While the heavy chain gene locus is rich in recombinational opportunities, it appears that the chances of assembling all of these segments correctly is diminished. Thus, intermediate and aberrant recombinations of these segments (V_H, D_H, and J_H) may occur even more frequently in the case of heavy chain gene rearrangements than in the case of light chain rearrangements. Whether the wastage of genetic material that occurs with such recombinations represents the cost of maintaining this wonderfully flexible system or in itself promotes the utilization of alternative gene segments, thus expanding antibody diversity, is an open question.

When active variable heavy (V_H) region genes and their products were compared with the parental germ line V_H gene regions from which they presumably originated, an element of somatic mutation was discovered. However, nucleic acid base changes that result in amino acid substitutions are not confined to the complementarity-determining regions (CDRs) felt to be immediately involved in antigen binding but are actually found scattered throughout the entire V_H region of an actively expressed gene. Of note is the much more frequent occurrence of such point mutations within the V_H regions associated with gamma and alpha heavy chains than with the variable portion of mu chains. Thus, the evidence suggests that the primary IgM response may utilize unmodified germ line V_H regions, while heavy chain class switching or continued gene usage might prompt somatic changes within the V_H region. Furthermore, different V_H gene segments with better antigen affinity can be recruited during the anamnestic response. Thus, in addition to somatic mutation, other mechanisms including clonal selection may play important roles in generating the higher affinity antibodies of the secondary IgG and IgA responses as compared to the lower-affinity primary IgM response.

GENERATION OF ANTIBODY DIVERSITY

Several genetic mechanisms appear to contribute to the generation of an individual's total repertoire of antibody specificities. First of all, both heavy and light chain genes utilize multiple alternative gene segments to assemble a complete variable region. For example, if each germ line V_H segment is capable of recombining with any D_H segment and that V_H/D_H complex with any of the available J_H regions, then an enormous amount of diversity can be created by chance recombinatorial joining. Additional amino acid variability can be further generated by allowing the frame of recombination to vary at the sites of juncture of heavy chain V_H/D_H, D_H/J_H, and light chain V_L/J_L segments. On top of this purely germ line contribution may be an additional component of somatic mutation. The D_H, J_H, and J_L regions in essence represent important "minigenes" that correspond to major portions of a hypervariable or complementarity-determining region (CDR_3) of heavy and light chain variable regions, respectively. However, no further gene subsegments corresponding to the other 2 markedly hypervariable portions of the variable region (CDR_1 and CDR_2) have been identified. Other genetic mechanisms, including gene conversion, may prove responsible for maintaining framework and hypervariable regions.

A given heavy chain produced initially by a B cell precursor should theoretically be able to associate with any of a multitude of kappa or lambda light chains the cell could produce. Because the configuration and specificity of the antigen-binding site is affected by both heavy and light chains, alternative combinations of heavy and light chains should markedly enhance diversity.

SEQUENTIAL ACTIVATION OF IMMUNOGLOBULIN GENES

During ontogeny, pre-B cells producing mu chain demonstrable in the cytoplasm but no light chain or surface immunoglobulin clearly precede the appearance of surface IgM-bearing B cells. At the immunoglobulin gene level, several stages of gene recombination have been identified within the B cell precursor series. Early B cell precursors can be identified in which only heavy chain gene recombina-

tions have occurred and all light chain genes remain in their germ line configurations. Heavy chain gene recombination itself often appears to be stepwise, in which the creation of D_H/J_H intermediate rearrangements precedes the later addition of V_H segments ($V_H/D_H J_H$). If the attempt at recombining a V_H, D_H, and J_H segment is complete, cytoplasmic mu chain will be produced; but if it is intermediate (D_H/J_H) or aberrant, no complete heavy chain will be synthesized. Other cells within the B cell developmental series have been observed which indicate that following heavy chain gene rearrangement, the first attempt at light chain gene rearrangement generally involves the kappa gene class as opposed to the lambda gene class. If a V_κ and a J_κ segment are effectively joined in a cell already possessing an effective $V_H/D_H/J_H$ recombination, a mature mu, kappa-bearing B cell will result. Not infrequently, however, both the maternal and paternal sets of kappa alleles actually delete at this stage of differentiation. This kappa gene loss is an evolutionarily conserved event uniformly mediated by a unique deleting element. The kappa deleting genetic element rearranges site—specifically with a recombinational signal (CACAGTG) to eliminate the C_κ segment. The same recombinational enzymes that assemble this gene appear also to destroy it and pave the way for lambda light chain usage. This leaves the cell with 2 sets of lambda genes to recombine in attempts to form a functional light chain. Accordingly, such lambda gene recombinations may be effective, resulting in a mu, lambda-bearing B cell, or might also be mistaken, retaining the cell within the pre-B cell series. This cascade of immunoglobulin gene recombinations, which moves from heavy chain genes to kappa genes and then to lambda, appears to be quite error-prone. Consequently, a sizable proportion of B cell precursors entering this pathway may waste all of their chances to form an effective heavy or light chain gene and be incapable of further expansion.

The expression of the gene for J chain, the 15,000-molecular-weight protein that links together IgM and IgA monomers, accompanies the expression of immunoglobulin. The human J chain gene is composed of 4 exons and does not rearrange during differentiation. J chain is expressed early at a pre-B cell stage in humans. The gene's transcription appears to be turned off during maturation at a nonsecretory stage of B cell development. It is once again actively transcribed in secretory B cells, including IgG producers, even though J chain protein is not utilized in secreted IgG.

HEAVY CHAIN CONSTANT REGION GENE STRUCTURE

The domain and hinge regions that can be identified within immunoglobulin heavy chain are each encoded as a distinct structural gene segment (exons) separated by intervening sequences (introns) at the DNA level. These constant region subunits are assembled together at the level of RNA by a splicing removal of the intervening sequences. The germ line organization and splicing pattern of the C_μ region is detailed in Fig 5–5. The other constant heavy chain gene regions are similarly designed. The universal RNA splicing mechanism that assembles these structural subunits depends upon the presence of donor (GT) and acceptor (AG) splice signals located at the respective ends of these intervening sequences. If such signals are not present, RNA splicing cannot occur, and such a gene segment might be nonfunctional and thus classified as a "pseudogene." Examples of such pseudogenes have now been found within various immunoglobulin variable, joining, and constant region gene segments.

IgM exists in 2 forms, either as a monomeric membrane-bound receptor on B cells or in a secreted pentameric form. Initial studies suggested that the mu chain portion of the membrane-bound IgM (μ_m) was larger, containing more hydrophobic amino acids at its carboxy-terminal end. Studies of DNA and RNA have shown the molecular basis for the mu membrane (μ_m) and mu-secreted (μ_s) forms of IgM. There are 2 distinct mRNAs, one for μ_m and one for μ_s, that are transcribed from a single C_μ gene locus composed of the 4 separated domains (Fig 5–5). However, the 20 hydrophilic carboxy-terminal amino acids of the μ_s chain are contributed by a short μ_s gene segment contiguous with the $C_\mu 4$ domain segment. In contrast, the 41 residues of the highly hydrophobic membranous portion of the μ_m chain are provided by 2 exons located 1850 base pairs to the 3′ side of the $C_\mu 4$ domain segment. Generation of this μ_m mRNA takes advantage of an alternative RNA donor splice site located at the boundary of the $C_\mu 4$ and μ_s segments. A splice between this site and an acceptor splice site at the 5′ side of the first μ_m exon actually deletes the structural μ_s gene segment and is responsible for joining the $C_\mu 4$ segment to the μ_m terminal segment (Fig 5–5). The choice between creating a μ_m versus a μ_s mRNA is probably dictated by the site of transcription termination and the addition of poly(A) to one of 2 alternative sites. If polyadenylation occurs at the site immediately 3′ to the $C_\mu 4$ segment, the μ_s mRNA would be produced. Alternatively, if the transcript is extended to the poly(A) site found 3′ to the μ_m exon, then RNA splicing would produce μ_m RNA. Thus, alternative modes of RNA splicing create 2 functionally distinct polypeptides from a single gene locus.

HEAVY CHAIN GENE ORDER & CLASS SWITCHING

An immature B lymphocyte bearing only surface IgM develops into a cell that simultaneously produces IgM and IgD and is subsequently capable of switching to the production of IgG, IgA, or IgE. Of central importance is the fact that each of these heavy chain classes is associated with the same variable heavy (V_H) region in a given cell. Establishing the order of the genes in the heavy chain constant region gene locus has helped elucidate the mechanism by which differ-

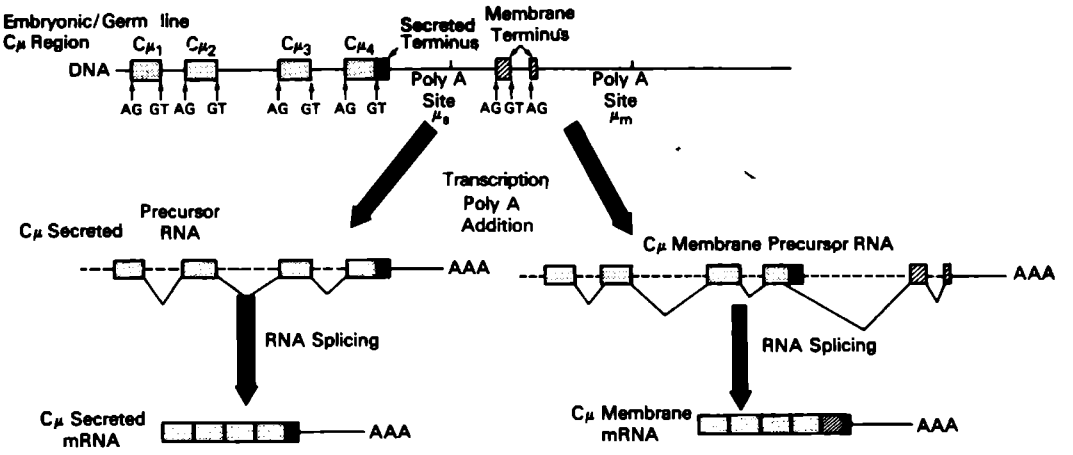

Figure 5–5. Schematic model for creating distinct secreted and membranous forms of IgM from a single constant region locus. Donor (GT) and acceptor (AG) splice sites for RNA splicing border the 4 separated C_μ domains. Alternative sites of poly(A) addition and RNA splicing result in different mRNAs containing either the secreted or the membrane terminus.

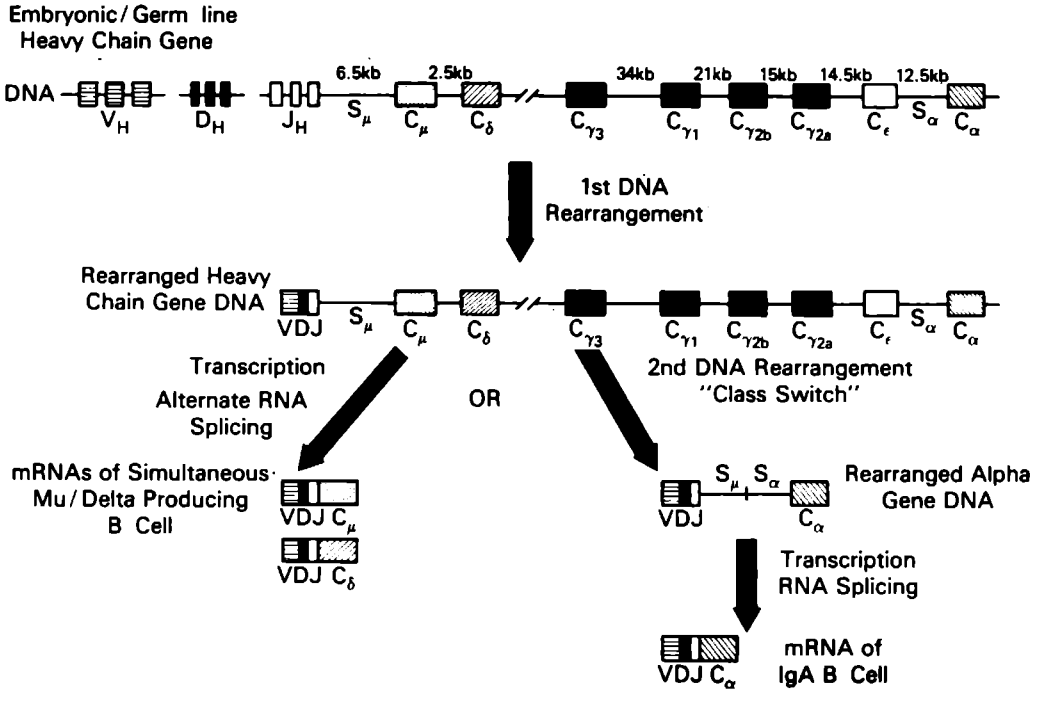

Figure 5–6. Schematic diagram of the mouse heavy chain gene locus, revealing the constant region gene order and spacing. Following the initial DNA rearrangement recombining a V_H, D_H, and J_H region, a B cell can utilize alternative sites of RNA splicing to simultaneously produce IgM and IgD. Alternatively, such a B cell can further differentiate and switch to production of another heavy chain class. For example, a second DNA recombination at the highly homologous switch sites (S_μ and S_α) in front of the C_μ and C_α genes would result in IgA production. Similar homologous switch sites (not shown here) are found in front of each of the constant regions.

six classes are produced. As can be seen in Fig 5–6, the mu constant (C_μ) region is closely associated with the delta constant (C_δ) region. Considerable space separates these 2 genes from the gamma constant (C_γ) region cluster, whose subclass members in the mouse appear in the order of $C_\gamma 3$, $C_\gamma 1$, $C_\gamma 2b$, and $C_\gamma 2a$. The epsilon constant region (C_ϵ) is located 5′ to the terminal alpha constant (C_α) region.

The close proximity of the C_μ and C_δ regions (only 2500 base pairs apart in the mouse) allows for the simultaneous production of IgM and IgD, which bear the same assembled variable region. Nuclear RNA transcripts from a recombined $V_H/D_H/J_H$ region might undergo differential processing at alternative RNA splice sites to connect a single $V_H/D_H/J_H$ recombined gene with either the C_μ or C_δ region (Fig 5–6). Such a mechanism would be quite analogous to that documented for the mu membrane versus mu-secreted forms of IgM. This could easily account for the capacity of a single lymphocyte to express both of these constant regions (μ and δ) without undergoing any further DNA rearrangements. Conversely, the gamma (C_γ) and alpha (C_α) constant regions are located a considerable distance 3′ to the mu constant (C_μ) region. Initial studies revealed a deletion of all constant regions located to the 5′ side of an expressed gamma region subclass or alpha constant region. This suggested that a DNA rearrangement event mediated class switching from mu to either the gamma or alpha constant regions. Nucleic acid sequence analysis has revealed the presence of switch regions located on the 5′ side of each constant region that appear to mediate these changes in heavy chain class production. The mu switch region on the 5′ side of the C_μ region is a 2000–3000 base pair region comprised of tandemly arranged short repetitive units which are themselves composed of quite homogeneous short base pair repeats of GAGCT and GGGT. Switch regions also occur in front of the other constant regions and are composed of similar repetitive units. The repetitive nature of these switch regions may promote homologous recombinations between the mu switch region and further 3′ switch regions. Although the exact site of recombination can vary, the consensus sequence of YAGGTTA has been noted near such recombinations and may be important in focusing the point of juncture. Such recombinations would result in a DNA rearrangement that is accompanied by deletion of the C_μ region and other intervening DNA. Most importantly, it would allow a new constant region to be transcribed with the preexisting $V_H/D_H/J_H$ recombined gene.

A TISSUE-SPECIFIC ENHANCER ELEMENT AUGMENTS IMMUNOGLOBULIN GENE TRANSCRIPTION

The series of DNA rearrangements discussed thus far assemble a single transcriptional unit responsible for the precursor RNA that encodes immunoglobulin heavy or light chains. The variable (V) region segments have routine eukaryotic promoter elements in their 5′ flanking sequences as well as other upstream elements that may influence their transcription. Effectively recombined V regions are already transcribed at low levels in B cell precursors and ultimately at extremely high rates (4 orders of magnitude greater) in plasma cells. At least part of this transcriptional increase is mediated by enhancer elements that are located downstream from the V region's classic promoter sequences. The immunoglobulin enhancers are quite similar to previously described viral elements and are capable of positively influencing a variety of linked genes when placed either upstream or downstream. In the case of the heavy chain, an enhancer element (E) is located between the J_H segments and the μ switch region (Fig 5–7). The κ locus appears to have a similar enhancer element located between J_κ and C_κ. Thus, a single downstream enhancer would have the ability to augment the transcriptional efficiency of any of the V regions once they were rearranged. In addition, the location of the enhancer element on the 5′ side of the μ switch region is also an efficient use of DNA. As can be seen in Fig 5–8, this single enhancer would be retained following a heavy chain class switch and thus could confer transcriptional competence to any of the heavy chain classes. A most important property of the immunoglobulin enhancer is its tissue specificity. It functions in lymphoid cells but not in other tissues such as fibroblasts and may well display stage-specific differences within B cell development. Such elements may prove to play profound roles in regulating normal as well as malignant growth and development.

CHROMOSOMAL TRANSLOCATIONS INVOLVING IMMUNOGLOBULIN GENE LOCI

Chromosomal translocations represent an additional DNA recombination that occurs near the various immunoglobulin gene loci in certain human B cell cancers. This rearrangement juxtaposes information originally located on separate chromosomes. One such translocation is characteristically found in Burkitt's lymphoma. The usual chromosomal aberration t(8;14) involves a reciprocal exchange of segments of chromosome 14 at band q32 and chromosome 8 at band q24 (Fig 5–8). Indeed, chromosomal in situ hybridization studies localized the heavy chain genes to 14q32 in humans. This association of immunoglobulin gene loci with chromosomal translocations was emphasized when variant forms of Burkitt's translocations involved chromosomes 2p11 and 22q11 (the location of κ and λ genes, respectively). Therefore, one side of this chromosomal recombination occurred at or near an immunoglobulin gene locus. Since these chromosomal translocations did not occur in normal B cells, it was possible that the locus at 8q24 which uniformly participated in this rearrangement might con-

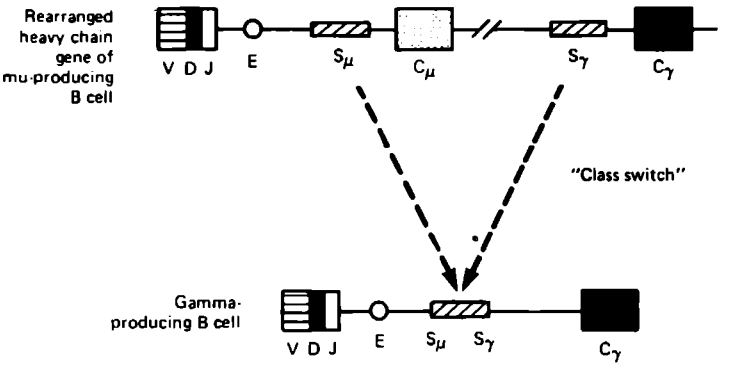

Figure 5–7. Schematic model displaying an enhancer element (E) strategically located between the J_H and μ switch (S_μ) regions. Because of its location, the enhancer would be retained following a class switch and be able to positively influence any heavy chain class selected.

tribute to the malignant phenotype. In fact, c-*myc*, a member of a candidate set of cancer-related genes known as cellular oncogenes, has been shown by chromosomal in situ hybridization to be located at 8q24 (Fig 5–8). In chickens, the c-*myc* cellular oncogene was known to be activated by the nearby insertion of avian leukosis virus and manifested as bursal lymphomas. In addition, mouse plasmacytomas manifest similar translocations between immunoglobulin gene loci and the c-*myc* locus. Examination of human Burkitt lymphoma cells revealed that the c-*myc* gene had actually moved to the $14q^+$ translocated chromosome (Fig 5–8). The exact recombinational breakpoint on the $14q^+$ chromosome between the heavy chain gene locus and the c-*myc* gene locus can vary considerably. The c-*myc* gene may be as close as the μ switch region in front of the C_μ region or can be a considerable distance upstream. The translocated c-*myc* gene has frequently been inserted in the opposite direction of the $C\mu$ region, and thus the RNA transcripts from this gene originate off of the opposite strand from the C_μ transcripts. This chromosomal break results in the reciprocal movement of a portion of chromosome 14, frequently bearing V_H regions and enhancer region to the $8q^-$ chromosome (Fig 5–8). The normal chromosome 14 in the cells is responsible for the production of IgM and thus contains an effective $V_H/D_H/J_H$ recombination. The normal chromosome 8 retains a germ line copy of c-*myc*, but the elevated levels of c-*myc* RNA are produced by the gene translocated to chromosome $14q^+$ Thus, the introduction of a cellular oncogene into an active locus such as the immunoglobulin genes results in an environmental change in the c-*myc* gene that may play an integral role in creating or maintaining a malignant state. The precise mechanism by which this oncogene shuffling participates in this cancer awaits the identification of the function of the c-*myc* gene product.

Importantly, other human lymphomas with a mature B cell phenotype also demonstrate characteristic chromosomal translocations that consistently involve the heavy chain gene at chromosome segment 14q32. Occasional chronic lymphocytic leukemias introduce chromosome segment 11q13 into the J_H region on chromosome 14. Moreover, the commonly occurring follicular lymphomas rearrange chromosome segment 18q21 into the J_H region in over 60% of cases. These rearrangements are focused within a small breakpoint cluster region on chromosome 18q21 next to a new transcriptional unit. Cloning the chromosomal breakpoints of these lymphomas has provided a bridge to identify new genes involved in B cell transformation. Furthermore, the lineage association of immunoglobulin gene loci with translocations in B cells predicts that the T cell receptor genes will mediate translocations in some T cell neoplasms. This thesis is already proving to be correct.

APPLICATION TO CLINICAL IMMUNOLOGY

While human B cells uniformly display the necessary recombinations of heavy and light chain genes required to synthesize immunoglobulin, human T cells retain their light and usually their heavy chain joining and constant region gene segments in the germ line configuration. This marked difference between B and T cells at the immunoglobulin gene level has proved useful in the determination of the cellular origin of human lymphoid malignancies lacking mature B or T cell surface markers. By such analysis, most cases of common "non-T, non-B" acute lymphocytic leukemias correspond to discrete stages of immunoglobulin gene recombination occurring within the B cell developmental series. Similarly, the lymphoid blast crisis phase of chronic myelogenous leukemia (CML) belongs to a B cell precursor stage of development that has proved capable of undergoing immunoglobulin gene rearrangements. In addition, the cells of contro-

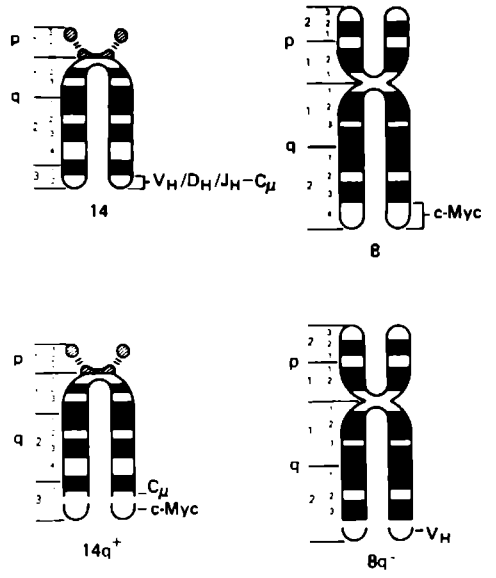

Figure 5–8. Schematic representation of a Burkitt lymphoma t(8;14) translocation detailing the normal and reciprocally translocated chromosomes. The normal chromosome 14 is responsible for the production of IgM and bears an effective $V_H/D_H/J_H$ rearrangement. The normal chromosome 8 retains a germ line c-*myc* gene, whereas the $14q^+$ chromosome has received the distal portion of chromosome 8 (8q24) bearing c-*myc* into band 14q32 that retains the C_μ region. The $8q^-$ chromosome at the breakpoint of 8q24 has reciprocally received a portion of chromosome 14 that includes the V_H gene segments.

versial origin in hairy cell leukemia demonstrate the patterns of immunoglobulin gene rearrangement and expression that establish this cancer as a genotypic B cell. Examining the configuration of both the immunoglobulin heavy and light chain genes as well as T cell receptor genes is proving indispensable in determining the cellular origin of solid neoplasms with large numbers of nonneoplastic infiltrating cells. The fact that a given cancer cell possesses unique, identifiable DNA rearrangements when compared with any other cell serves as a powerful tumor-specific clonal marker. The use of gene rearrangements as a sensitive as well as specific tumor marker has enhanced the ability to identify persistent tumor following therapy and facilitate the early detection of disease.

Human heavy chain disease disorders represent malignant expansions of cells that frequently secrete only a heavy chain which is itself defective, often missing an entire domain or hinge region. Examination of the DNA and RNA that code for the affected heavy chain immunoglobulin in such cells has provided molecular genetic explanations for these missing segments. A DNA insertion/deletion event has been discovered in mu heavy chain disease that eliminates a J_H donor splice site on the expressed heavy chain allele. This forces an alternative RNA processing that splices leader sequence information directly to C_μ. The subsequent immunoglobulin is a truncated surface and secreted mu chain without a variable region and thus lacks the capacity to bind light chain.

Analysis of the structure and functional capacity of immunoglobulin genes within a variety of humoral immunodeficiency syndromes may pinpoint the inherited defect within some of these individuals. An analysis of the human heavy chain constant regions indicated that a large distal (3′) gene duplication event has expanded the heavy chain genes in humans (Fig 5–9). This event appears to have duplicated two C_γ units, a C_ϵ unit, and a C_α unit. The first epsilon region ($C_{\epsilon\psi}$) is a pseudogene lacking the first 2 domains. Humans are known to express the four C_γ genes and $C_{\alpha 1}$ and $C_{\alpha 2}$ that are shown in Fig 5–9. The apparent order of these human constant regions is pertinent when considering some of the humoral immunodeficiency syndromes. It is curious that a number of patients with IgA deficiency as a result of several different disease states have also been noted to have reduced or absent IgE and occasionally IgG2 and IgG4. These underproduced heavy chain isotypes are the most 3′-located genes in the complex (Fig 5–9). A small percentage of these cases have actually lost these 3′ located genes from unequal meiotic crossover events, resulting in constant region deletions. Most of these patients retain these genes, yet the 3′ location of these regions raises the tantalizing possibility that a defect might exist in the capacity to switch to or appropriately express these distal constant regions. Thus, the molecular genetic description of the immunoglobulin gene loci has contributed an enormous amount of information concerning not only the generation of antibody diversity but a number of immunopathologic states as well.

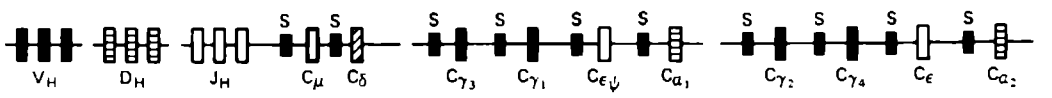

Figure 5–9. Schematic representation of the human germ line heavy chain constant region gene order. Each constant region (C) has a switch region (S) located on its 5′ side. The first C_ϵ region is a nonfunctional pseudogene (ψ)

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6 The Human Major Histocompatibility HLA Complex

Benjamin D. Schwartz, MD, PhD

The discovery of the human major histocompatibility complex (MHC) dates from the mid 1950s, when leukoagglutinating antibodies were first found in the sera of multiply transfused patients and in the sera of 20–30% of multiparous women. Analysis of the reaction patterns of these antisera indicated that each antiserum gave a positive reaction with the cells of some but not all individuals and that different antisera reacted with the cells of different but overlapping populations of individuals. This pattern suggested that these antisera were detecting **alloantigens** (ie, antigens present on the cells of some individuals of a given species) which were the products of a polymorphic genetic locus.

The role of these antigens in determining the success of tissue and organ transplants was soon appreciated and provided the initial impetus for studying the genes that determine human leukocyte antigens (HLA). The advent of microcytotoxicity testing to type for specific HLA antigens and the use of computer technology for the codification of the reaction patterns of literally thousands of anti-HLA alloantisera have made possible the delineation of the HLA system. An International Workshop meets every 2–3 years to update the description of the organization and nomenclature of the HLA complex based on findings since the previous Workshop.

In 1973, certain HLA antigens were found to be associated with specific diseases in a high proportion of cases. In addition, in the past decade, it was realized that the HLA complex regulates several aspects of the human immune response. These findings provided a second impetus for the study of the HLA complex. The recent application of recombinant DNA technology to the HLA complex has allowed delineation of the HLA genes and the amino acid sequence of many HLA molecules.

NOMENCLATURE & GENETIC ORGANIZATION OF THE HLA SYSTEM

The nomenclature of the HLA system is devised by the HLA Nomenclature Committee under the auspices of the World Health Organization. The entire histocompatibility complex is termed the HLA complex. It occupies a segment of approximately 4 centimorgans* (cM) on the short arm of **chromosome 6.** Fig 6–1 schematically depicts our current concept of the HLA complex, showing the genetic regions containing the HLA loci. (A locus is the position on the chromosome where a given gene may be found.) The position of the regions with respect to one another and to the centromere, as well as the estimated map distances between regions, is included. The 7 genetic loci presently officially recognized by the HLA Nomenclature Committee are HLA-A, HLA-B, and HLA-C, which determine class I antigens; and HLA-D, HLA-DR (HLA-D-related), HLA-DQ (formerly DC, MB, DS), and HLA-DP (formerly SB), which determine class II antigens (see below for explanation of classes I and II). Results from studies of the molecular biology of the HLA complex may lead to the official recognition of additional loci.

Several additional genetic regions have been mapped to the HLA complex. The complement region, which has been mapped between the HLA-B and HLA-DR regions, contains genes determining the second and fourth components (C2 and C4) of the classic complement pathway and properdin factor B (BF) of the alternative pathway.

At each locus, one of several alternative forms (alleles) of a gene may be found. Officially recognized alleles at each locus are designated by the locus and a number; thus, HLA-A1 is the 1 allele at the HLA-A locus. Alleles that have been tentatively assigned to a given locus but are not yet officially recognized are designated by a *w* (for "workshop") placed before the number, eg, HLA-DRw1. Official recognition results in the elimination of the *w*, eg, HLA-DR1.

The HLA system is extremely polymorphic, having multiple different alleles at each known locus. For example, there are at least 23 distinct alleles at the HLA-A locus and at least 47 distinct alleles at the HLA-B locus. Each allele determines a product. The products of the HLA-A, -B, -C, -D, -DR, -DQ, and -DP alleles are cell surface molecules that bear the antigenic determinants. With the exception of HLA-D, which is detected by a mixed leukocyte reaction

*A centimorgan is a unit of physical map distance on a chromosome equivalent to a 1% frequency of recombination between linked genes. It is also called a map unit.

Supported in part by NIH grants AI-18925 and AI-15322.

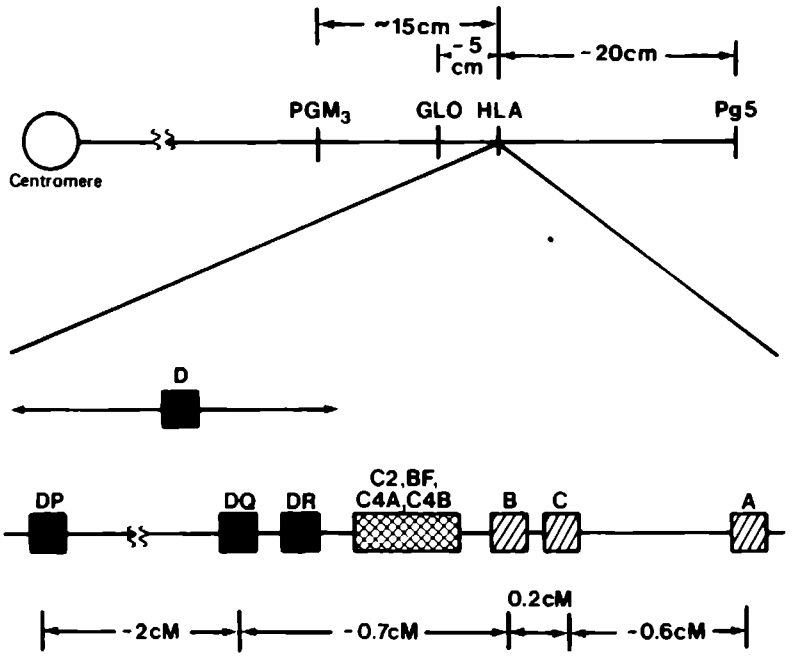

Figure 6–1. The HLA complex on the short arm of chromosome 6. In the upper part of the figure, the position of the HLA complex relative to other markers (PGM_3 = phosphoglucomutase 3; GLO = glyoxylase; Pg5 = urinary pepsinogen) on the short arm of chromosome 6 is shown. Approximate distances are given in centimorgans (cM). The lower part of the figure displays an expanded version of the HLA complex. Class I loci are striped, class II loci are solid, and complement loci are stippled. The lymphocyte-activating determinants known as HLA-D are present on molecules determined by several class II loci.

(MLR), and DP, which is identified by **primed lymphocyte typing (PLT),** all of the cell surface antigens are detected serologically, usually by microcytotoxicity. The products of the C2, C4, and BF loci are soluble serum proteins that can be detected serologically or functionally.

The same term is used to designate the HLA allele and its product, the HLA antigen. HLA antigens found on the molecule determined by a single allele (and no other) are termed HLA private antigens. In contrast, HLA public antigens are determinants common to several HLA molecules each of which bears a distinct HLA private antigen. HLA-Bw4 and -Bw6 are the best-known examples of HLA public antigens. The entire listing of officially and tentatively recognized HLA antigens determined by the HLA-A, -B, -C, -D, -DR, -DQ, and -DP loci is presented in Table 6–1. The distribution of HLA-Bw4 and -Bw6 on HLA-B antigens is presented in Table 6–2.

In several instances, HLA antigens initially thought to be single private HLA antigens have been subsequently found to be a group of 2 or 3 closely related HLA antigens, each of narrower specificity. These latter antigens are termed "splits" of the original broad specificity. In Table 6–1, HLA antigens that are splits are followed in parentheses by the original broad antigen of which they are splits. Thus, for example, HLA-A25(10) and HLA-A26(10) indicate that HLA-A25 and -A26 are splits of HLA-A10. HLA-A10 could thus be considered a public antigen on the HLA molecules bearing the private HLA-A25 and -A26 antigens. Table 6–3 is a listing of the currently recognized splits of the broad specificities. Recently, a monoclonal antibody has been found that defines a split of HLA-B27, and cytotoxic T cell clones have been produced that recognize splits of other HLA antigens. Biochemical analysis showed these splits to be structural variants.

Conversely, **HLA private antigens** can be organized into groups based on apparent serologic cross-reactivity between members of the group. These groups are termed **cross-reactive groups (CREGs).** Thus, for example, the B7-CREG includes HLA-B7, Bw22 (subsequently split into Bw54, Bw55, and Bw56), B27, B40 (subsequently split into Bw60 and Bw61), and Bw42. For at least 3 of the CREGs (B5-CREG, B7-CREG, and B15/B17-CREG), the basis for the cross-reactivity has been demonstrated to be a public HLA antigen common to all members of the CREG, and it is assumed that public antigens will also explain the cross-reactivity between members of other CREGs. A listing of some CREGs and their members is presented in Table 6–4.

The organization of the HLA-D region has recently been clarified. The DQ (formerly MB, DC) antigens are detected serologically and are distinct from HLA-

Table 6–1. Complete listing of recognized HLA antigens.

HLA-A	HLA-B		HLA-C	HLA-D	HLA-DR	HLA-DQ	HLA-DP
A1	Bw4	Bw47	Cw1	Dw1	DR1	DQw1	DPw1
A2	B5	Bw48	Cw2	Dw2	DR2	DQw2	DPw2
A3	Bw6	B49(21)	Cw3	Dw3	DR3	DQw3	DPw3
A9	B7	Bw50(21)	Cw4	Dw4	DR4		DPw4
A10	B8	B51(5)	Cw5	Dw5	DR5		DPw5
A11	B12	Bw52(5)	Cw6	Dw6	DRw6		DPw6
Aw19	B13	Bw53	Cw7	Dw7	DR7		
A23(9)	B14	Bw54(w22)	Cw8	Dw8	DRw8		
A24(9)	B15	Bw55(w22)		Dw9	DRw9		
A25(10)	B16	Bw56(w22)		Dw10	DRw10		
A26(10)	B17	Bw57(17)		Dw11(w7)	DRw11(5)		
A28	B18	Bw58(17)		Dw12	DRw12(5)		
A29(w19)	B21	Bw59		Dw13	DRw13(w6)		
A30(w19)	Bw22	Bw60(40)		Dw14	DRw14(w6)		
A31(w19)	B27	Bw61(40)		Dw15			
A32(w19)	B35	Bw62(15)		Dw16	DRw52		
Aw33(w19)	B37	Bw63(15)		Dw17(w7)	DRw53		
Aw34(10)	B38(16)	Bw64(14)		Dw18(w6)			
Aw36	B39(16)	Bw65(14)		Dw19(w6)			
Aw43	B40	Bw67					
Aw66(10)	Bw41	Bw70					
Aw68(28)	Bw42	Bw71(w70)					
Aw69(28)	B44(12)	Bw72(w70)					
	B45(12)	Bw73					
	Bw46						

DR antigens, but because of linkage disequilibrium (see below) they are associated with groups of HLA-DR antigens. Thus, for example, DQ1 is associated with HLA-DR1, -DR2, and -DRw6. DRw52 (formerly MT2) and DRw53 (formerly MT3) were also associated with groups of HLA-DR molecules such that DRw52 was associated with DR3, DR5, DRw6, and DRw8; and DRw53 was associated with DR4, DR7, and DRw9. It is now clear, as is reflected by the nomenclature change, that these antigens are on molecules determined by the DR subregion. However, because multiple DR molecules are determined by the DR subregion (see below), it is not yet firmly established if the DRw52 and DRw53 determinants reside on one or more DR molecules. A listing of the DRw52, DRw53, and DQ antigens and their associations with HLA-DR antigens is given in Table 6–5.

The DP (formerly SB) antigens are identified by primed lymphocyte typing (see below). The reagents used to define the DP antigens are sets of cryopreserved primed lymphocytes from different donor combinations and include 2 reagents to define each of the DP antigens. Currently, six DP antigens have been defined (DPw1–DPw6). No associations have been found between the DP antigens and the DR or DQ antigens.

The complement components determined by the HLA-linked complement loci also display polymorphism. There are 4 alleles determining the 4 alternative forms of properdin factor B that can be distin-

Table 6–2. Distribution of HLA-Bw4 and -Bw6 on the HLA-B antigens.

Public Antigen	HLA-B Antigens on Which It Is Found
Bw4	B13, B27, B37, B38(w16), Bw44(w12), Bw47, Bw49(w21), Bw51(5), Bw52(5), Bw53, Bw57(17), Bw58(17), Bw59, Bw63(15)
Bw6	B7, B8, B14, B18, Bw35, Bw39(w16), Bw41, Bw42, Bw45(12), Bw46, Bw48, Bw50(w21), Bw54(w22), Bw55(w22), Bw56(w22), Bw60(40), Bw61(40), Bw62(15), Bw64(14), Bw65(14), Bw67, Bw71(70), Bw72(70), Bw73

Table 6–3. HLA antigen "splits."

Original Broad Specificity	Splits
A9	A23, A24
A10	A25, A26, Aw34, Aw66
Aw19	A29, A30, A31, A32, Aw33
A28	Aw68, Aw69
B5	B51, Bw52
B12	B44, B45
B14	Bw64, Bw65
B15	Bw62, Bw63
B16	B38, B39
B17	Bw57, Bw58
B21	B49, Bw50
Bw22	Bw54, Bw55, Bw56
B40	Bw60, Bw61
Bw70	Bw71, Bw72
DR5	DRw11, DRw12
DRw6	DRw13, DRw14
Dw6	Dw18, Dw19
Dw7	Dw11, Dw17

Table 6–4. Some HLA CREGs and their members.

HLA CREG	CREG Members
A1-CREG	A1, A3, A11, Aw36
A2-CREG	A2, A28
B5-CREG	Bw51(5), Bw52(5), Bw62(15), Bw63(15), B18, Bw35
B7-CREG	B7, B27, Bw54(w22), Bw55(w22), Bw56(w22), Bw60(40), Bw61(40), Bw42
B15-CREG	Bw62(15), Bw63(15), Bw57(17), Bw58(17)

guished by their electrophoretic mobility: a common fast form BF*F, a common slow form BF*S, a rare fast form BF*F1, and a rare slow form BF*S1. There are C2 alleles determining the 2 common forms of C2—C2*C and C2*A—and a rare deficiency allele C2*QO. The C4 locus has actually been duplicated, so that there are 2 distinct C4 genetic loci, designated C4A (formerly Rogers), which determines the electrophoretically more acidic group of C4 components; and C4B (formerly Chido), which determines the electrophoretically more basic group of C4 components. There are 7 common structural alleles and one deficiency allele at the C4A locus and 3 common structural alleles and one deficiency allele at the C4B locus. Table 6–6 presents a listing of the known common alleles at each of the HLA-linked complement loci.

Haplotype

Because of their close linkage, the combination of alleles at each locus on a single chromosome is usually inherited as a unit. This unit is referred to as the haplotype. Since we inherit one chromosome from each parent, we have two HLA haplotypes. Because all HLA genes are codominant, both alleles at a given HLA locus are expressed, and 2 complete sets of HLA antigens can be detected on cells. By simple mendelian inheritance, there is a 25% chance that 2 siblings will share both haplotypes, a 50% chance that they will share one haplotype, and a 25% chance that they will share no haplotype and will be completely HLA incompatible (Fig 6–2).

Linkage Disequilibrium

Owing to random matings, the frequency of finding a given allele at one HLA locus with a given allele at a second HLA locus should simply be the product of the frequencies of each allele in the population. However,

Table 6–5. DQ-, DRw52-, and DRw53-associated HLA-DR antigens.

DQw1	DQw2	DQw3	DRw52	DRw53
DR1	DR3	DR4	DR3	DR4
DR2	DR7	DR5	DR5	DR7
DRw6			DRw6	DRw9
DRw10			DRw11(5)	
			DRw12(5)	
			DRw13(w6)	
			DRw14(w6)	

Table 6–6. Common alleles at the HLA-linked complement loci.

BF	C2	C4A	C4B
BF*F	C2*C	C4A*1	C4B*1
BF*S	C2*A	C4A*2	C4B*2
BF*F1	C2*QO	C4A*3	C4B*3
BF*S1		C4A*4	C4B*QO
		C4A*5	
		C4A*6	
		C4A*7	
		C4A*QO	

certain combinations of alleles are found with a frequency far exceeding that expected. This phenomenon is termed "linkage disequilibrium" and is quantitated as the difference (Δ) between the observed and expected frequencies. As an example, the HLA-B8 allele and the HLA-DR3 allele are found in the North American white population with frequencies of 0.09 and 0.12, respectively. Thus, the expected frequency with which the HLA-B8-DR3 haplotype should be found is 0.09×0.12, or 0.0108. However, this haplotype is found with a frequency of approximately 0.0740, almost 7 times the expected frequency, for a Δ of $0.740 - 0.0108 = 0.0632$. Table 6–7 lists some common examples of linkage disequilibrium. Several hypotheses have been offered in an attempt to explain the phenomenon of linkage disequilibrium, including (1) a selective advantage of a given haplotype, (2) migration and admixture of 2 populations, (3) inbreeding, and (4) random drift.

THE HLA ANTIGENS

Based on their tissue distribution and structure, HLA antigens have been divided into 2 classes. **Class**

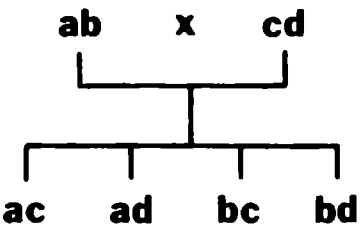

Figure 6–2. Inheritance of HLA haplotypes. A haplotype is the combination of alleles at each locus on a single chromosome that is inherited as a unit. Haplotype designations in the figure are given as a, b, c, and d. The maternal haplotypes are a and b, and the paternal haplotypes are c and d. Offspring of this mating (ab × cd) inherit one of the 2 possible haplotypes from each parent and so will have haplotypes ad, ab, bc, and bd. There is a 25% chance that 2 offspring will be HLA-identical (eg, ac and ac), a 25% chance that they will be totally HLA-nonidentical (eg ac and bd); and a 50% chance that they will be HLA-semi-identical (eg, ac and ad).

Table 6–7. Examples of linkage disequilibrium in Caucasians.

Haplotypes	Δ (X 10^3)
HLA-A1, B8	53.2
HLA-A2, B44	14.8
HLA-B27, Cw1	9.0
HLA-B27, Cw2	19.9
HLA-B7, DR2	36.8
HLA-B8, DR3	61.3
HLA-DR2, DQw1	93.6
HLA-DR4, DQw3	87.5

I antigens, also termed the classic histocompatibility antigens, include the HLA-A, -B, and -C antigens. **Class II antigens,** also termed Ia antigens, include the HLA-D, -DR, -DQ, and -DP antigens. **Class III antigens** include the BF, C2, and C4 components of complement and are discussed in Chapter 10.

HLA Typing

A. Class I Antigens: The HLA class I antigens are all defined by serologic reactions, and typing for these antigens is therefore performed using standard serologic techniques. Typing sera are obtained chiefly from multiparous women. These sera tend to have relatively high titers of antibodies directed against a limited number of HLA determinants, since in most cases the woman has been repeatedly immunized with the HLA antigens of a single individual—the father of her children—which are present on the fetuses she carries. Many attempts have been made to produce monoclonal antibodies with high titer and enough specificity for use as typing reagents. Recently, a mouse anti-HLA-B27 monoclonal reagent has been reported. The most widely used method for HLA typing is lymphocyte microcytotoxicity assay (Fig 6–3). Multiple antisera against HLA-A, -B, and -C antigens are placed in the microwells of a typing tray, and the trays are then frozen until needed. For typing, 1000–2000 peripheral blood lymphocytes are added to each microwell. After a short period of incubation, complement is added, and incubation continues. Finally, a vital dye such as eosin is added. Under phase microscopy, cells lysed by an antiserum and complement take up the dye and appear red, whereas live cells exclude the dye and remain unstained. The percentage of cells lysed by each antiserum can be determined, and the HLA-A, -B, and C phenotype can be assigned on the basis of the reaction patterns.

B. Class II Antigens: HLA-DR and -DQ antigens are typed by procedures virtually identical to those just described, with the exception that the typing is performed on purified populations of B lymphocytes. Alternatively, a double staining procedure is

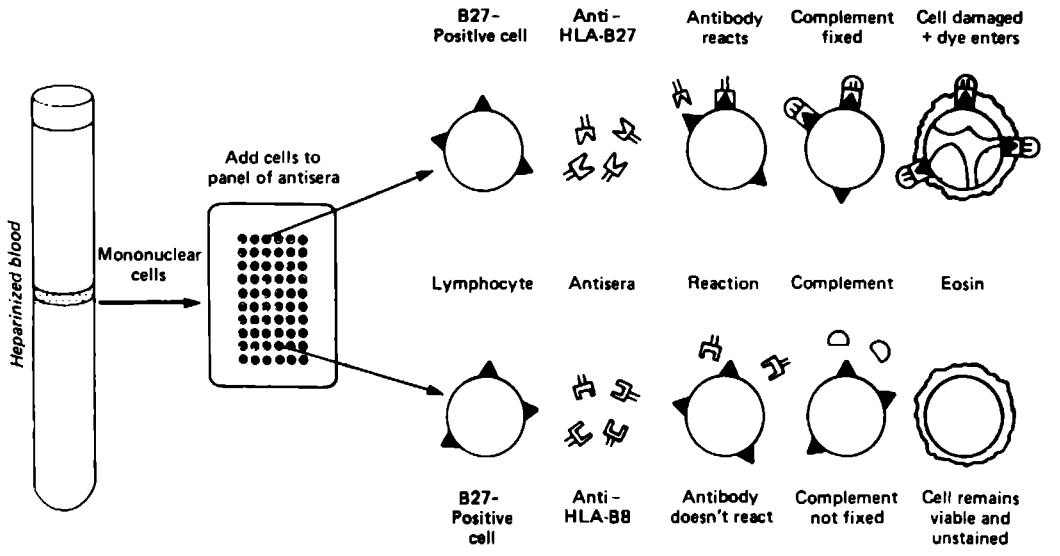

Figure 6–3. Microcytotoxicity testing for HLA antigens. The patient's blood is heparinized, and the mononuclear cells (lymphocytes and monocytes) are purified by Ficoll-Hypaque gradient centrifugation. The cells are then added to the wells of a microtiter tray containing antisera to HLA antigens. Illustrative examples of a B27-positive cell in wells with anti-B27 antisera (upper part) and anti-B8 antisera (lower part) are shown. Anti-B27 antibodies bind to the HLA-B27 antigens on the cell surface, fix complement, and cause the cell to lyse. Eosin then enters the cell, and the cell appears red under phase microscopy. In contrast, anti-B8 antibodies cannot bind to the cell; complement is not fixed; no lysis ensues; and the live cell excludes eosin and appears unstained under phase microscopy. The reaction patterns allow the cell to be typed as HLA-B27-positive, -B8-negative. This assay is used to type all HLA class I molecules and, with some modifications, to type HLA-DR and -DQ antigens.

used that allows B cells to be distinguished from T cells. The typing sera are pretested to make certain that they cannot be detecting HLA-A, -B, or -C antigens.

The HLA-D antigens are defined and typed by a mixed leukocyte reaction (MLR). In this test, lymphocytes from HLA-D-different individuals mixed in in vitro culture will manifest blast transformation, DNA synthesis, and proliferation in response to the foreign HLA-D antigens on the cells from the other individual. For HLA-D typing (Fig 6–4), a panel of **HLA-D-homozygous typing cells (HTC)** representing all known HLA-D types is used. These "stimulator" cells are irradiated or treated with mitomycin to prevent their DNA synthesis and proliferation in response to the unknown cells, so that a one-way MLR results. The "responder" lymphocytes of the individual to be typed are incubated in microculture with the HTC for 5 days. ^{3}H-thymidine is then added for an additional 12–18 hours, and the incorporation of ^{3}H-thymidine into DNA is used as a measure of DNA synthesis. If the unknown responder cells synthesize DNA after incubation with a particular HTC, then it is concluded that the unknown cells do not possess the same HLA-D type as the HTC. If the unknown responder cells show no DNA synthesis, then it is concluded that they do possess the same HLA-D type as the HTC. If only one HLA-D type can be assigned, it is possible to determine if the unknown cell is heterozygous or homozygous for that HLA-D type by reversing the one-way MLR. The unknown cells are irradiated or treated with mitomycin and used as stimulator cells. The ability of the HTC, which shares the HLA-D type with the unknown, to synthesize DNA in response to the unknown cell is determined. A lack of DNA synthesis indicates that the unknown cell is HLA-D-homozygous. DNA synthesis by the HTC indicates that it is recognizing a foreign HLA-D antigen and therefore that the unknown cell is HLA-D-heterozygous with one allele not typeable with the HTC panel used.

The DP antigens are identified and typed by **primed lymphocyte typing (PLT)** (Fig 6–5). A panel of responding lymphocytes is first primed by stimulator cells matched for HLA-A, -B, -C, -D, -DR, and -DQ antigens for approximately 10 days in an MLR, and is cryopreserved. Unknown cells are irradiated or treated with mitomycin C and tested for their ability to restimulate the primed responding lymphocytes. The unknown and primed responding lymphocytes are incubated in microculture for 30 hours, ^{3}H-thymidine is added for an additional 12–18 hours, and the incorporation of ^{3}H-thymidine into DNA by the primed lymphocytes is determined. Currently, a set of 2 different responding lymphocytes is used to define each DP antigen. DNA synthesis by a set of responding lymphocytes indicates that the unknown cell has the DP antigen defined by that set.

The HLA type of an individual is usually given as the phenotype and designates all HLA antigens possessed by the individual, eg, HLA-A1, -A2, -B7, -B12, -Cw1, -Cw2, -Dw2, -Dw3, -DR2, -DR3, -DQw1, -DQw2, -DRw52, -DPw1, and -DPw2. To

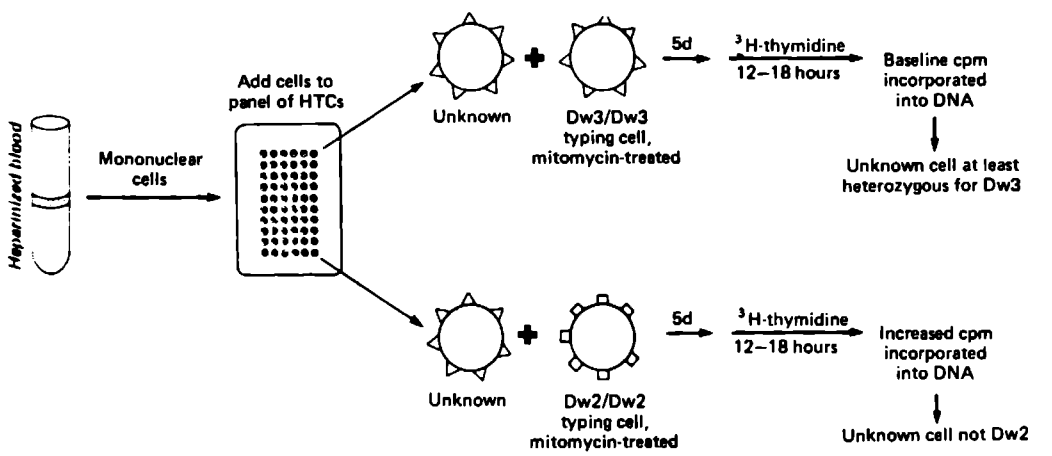

Figure 6–4. HLA-D typing by one-way mixed leukocyte reactions using homozygous typing cells. Mononuclear cells are prepared as described in the legend to Fig 6–3 and added to the wells of a microculture plate containing a panel of mitomycin-treated homozygous typing cells (HTCs) used as stimulator cells. Illustrative examples are given for the unknown responder cells (which are Dw3-positive) reacting with a Dw3/Dw3 HTC (upper part) and with a Dw2/Dw2 HTC (lower part). The cells are incubated for 5 days, after which ^{3}H-thymidine is added and incubation continued an additional 12–18 hours. The amount of ^{3}H-thymidine incorporated by the unknown cells into newly synthesized DNA is then determined. In the upper part of the figure, the unknown cell (which is Dw3-positive) does not recognize the Dw3/Dw3 HTC as foreign, does not proliferate, and does not incorporate ^{3}H-thymidine into DNA. In contrast (lower part), the unknown cell does recognize the Dw2/Dw2 HTC as foreign, does proliferate, and does incorporate ^{3}H-thymidine into DNA. The reaction patterns allow the cell to be typed as Dw3-positive, Dw2-negative.

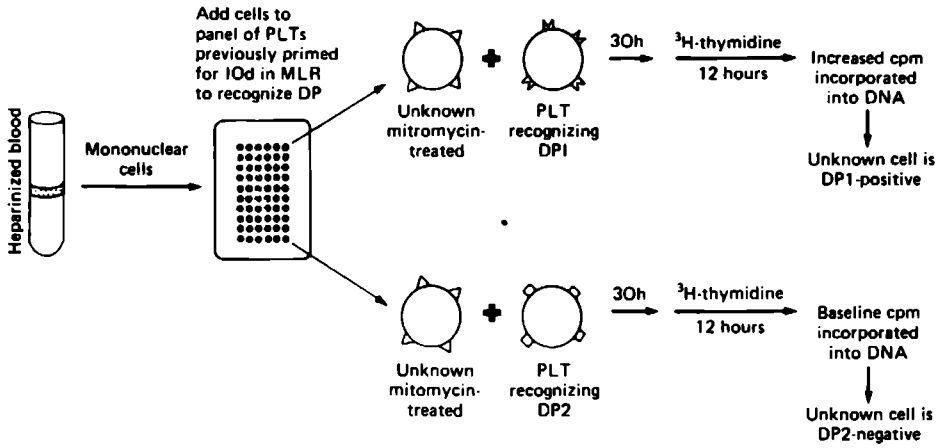

Figure 6–5. DP antigen typing by primed lymphocyte testing. Unknown mononuclear cells are prepared as in the legend to Fig 6–3 and treated with mitomycin. They are then added to the wells of a microculture tray containing lymphocytes previously primed in a 10-day mixed lymphocyte reaction with mitomycin-treated stimulator cells matched for HLA-A, -B, -C, -D, -DR, and -DQ antigens. The primed cells are known to recognize DP antigens. The cells are incubated for 30 hours, after which ^{3}H-thymidine is added for an additional 12 hours. The amount of ^{3}H-thymidine incorporated by the primed lymphocytes into newly synthesized DNA is then determined. Illustrative examples are given for an unknown cell (which is DP1-positive) incubated with a primed lymphocyte that recognizes DP1 (upper part) and with a primed lymphocyte that recognizes DP2 (lower part). In the upper part, the primed lymphocyte responds to DP1 and incorporates ^{3}H-thymidine into DNA. In the lower part, the primed lymphocyte cannot respond to DP1 and does not incorporate ^{3}H-thymidine into DNA. The reaction patterns allow the cell to be typed as DP1-positive, DP2-negative.

determine the HLA genotype of the individual, ie, which antigens are determined by which haplotype. family studies are necessary.

C. Blanks: In general, because we each possess 2 haplotypes and because HLA antigen expression is codominant, it is possible to type 2 antigens determined at a particular locus. Occasionally, only one antigen determined at a particular locus can be typed. The HLA type at that locus would then be identified as the typed HLA antigen and a "blank," eg, HLA-B27, B– or HLA-B27,–. In this situation, the individual may either be homozygous for the typed antigen or may have an antigen that cannot be typed by available reagents. These possibilities can usually be distinguished by family studies.

D. Future HLA Typing: There has been increasing interest in HLA typing at the DNA level by restriction endonuclease digestion. The pattern of restriction endonuclease fragments for a given HLA gene is dependent on the HLA gene, the restriction endonuclease used, and the probe employed. Eventually, it should be possible to correlate a given set of restriction endonuclease fragment patterns with a given HLA gene and thus to perform HLA typing at the DNA level.

Uses of HLA Typing

HLA typing is used primarily for a determination of HLA compatibility prior to transplantation, for paternity testing, for anthropologic studies, and for establishing HLA-disease associations.

HLA typing was first done to identify HLA-compatible or partially compatible donors and recipients for organ transplantation. The broadest generalization which can be made regarding HLA compatibility and transplantation is that results with closely related living donors matched with the recipient for one or both haplotypes are superior to those obtained with unrelated cadaveric donors matched for a similar number of HLA antigens. This finding derives from the fact that matching for the known HLA determinants within a nuclear family almost always assures compatibility for all gene products of the entire HLA complex. Usually, the serologically determined class I and class II antigens are typed, and the cells of the donor and recipient are mixed in an MLR.

Because of the relatively low frequency of a given HLA antigen and the even lower frequency of a given HLA haplotype, HLA typing has been useful in paternity testing. HLA typing is recommended as the next step if simple red cell typing does not exclude the putative father as the biologic father. If a putative father and the child share an HLA haplotype, the probability is high that the putative father is also the biologic father, but this contention cannot be considered proved. However, HLA typing demonstrating that the putative father and child do not share any haplotype is usually accepted by the courts as excluding the possibility that a given male is the father.

The finding that HLA types vary widely among different ethnic populations has allowed anthropologists to establish or confirm data regarding interrelationships among populations and migration patterns. For example, the finding that HLA-B27 is found in 8% of

American whites and 2% of American blacks but is virtually absent from African blacks suggests that the presence of HLA-B27 in American blacks results from an admixture of the American white and African black gene pools.

Finally, HLA typing has been used to establish HLA-disease associations, which are discussed below.

Tissue Distribution, Structure, & Function

A. Class I Antigens: The HLA-A, -B, and -C antigens are found on virtually every human cell (Table 6–8). Structurally, the class I antigens are found on a 2-chain molecule that consists of a polymorphic glycoprotein with a molecular weight of 44,000, determined by genes in the HLA complex, in noncovalent association with a nonpolymorphic 12,000-MW protein, β_2-microglobulin, determined by a gene on chromosome 15 (Fig 6–6). The entire molecule is anchored in the cell membrane by the 44,000-MW chain. Based on extensive structural studies, including determination of the entire amino acid sequences of several HLA 44,000-MW chains, it is known that the 44,000-MW chain contains 338 amino acid residues and can be divided into 3 regions. Starting at the N-terminal end of the molecule, these regions are an extracellular hydrophilic region (residues 1–281), a transmembrane hydrophobic region (residues 282–306), and an intracellular hydrophilic region (residues 307–338). The extracellular hydrophilic region in turn can be divided into 3 domains composed of amino acid residues 1–90, 91–180, and 181–271, respectively. The N-terminal domain (residues 1–90) bears the attachment site for the oligosaccharide side chain. The second and third domains (residues 91–180 and 181–271, respectively) each contain a disulfide loop reminiscent of those seen in immunoglobulin. Based on comparison of the amino acid sequences of several HLA antigens, it is clear that the HLA-antigenic determinants reside in the first or second (or both) of these hydrophilic domains. The 24 residues that comprise the hydrophobic transmembrane region render it long enough to span the hydrocarbon core of the lipid bilayer. The intracellular hydrophilic region can be phosphorylated, and it has been postulated that this reaction may allow extracellular signals to be transmitted to the interior of the cell.

The structure of a gene determining a class I antigen is shown in Fig 6–7. It consists of 7 exons, or translated regions of DNA, separated by introns, or untranslated regions of DNA. The second, third, and fourth exons correspond to the first, second, and third domains, respectively, of the 44,000-MW chain.

Much of what we know regarding the function of HLA antigens is based on the functions that have been demonstrated for major histocompatibility antigens in other species. Class I antigens are the principal antigens recognized by the host during tissue graft rejection. In cell-mediated cytolysis, the in vitro correlate of graft rejection, the class I antigens are the target antigens recognized by the killer T lymphocytes. Both private and public HLA antigens can be recognized independently by these T cells. The true physiologic role of class I histocompatibility, however, is probably related to the phenomenon of histocompatibility restriction of cell-mediated lysis (CML) of virus-infected and minor histocompatibility antigen-bearing cells. When T lymphocytes are exposed to a viral (or minor histocompatibility) antigen, they will recognize it in the context of a class I antigen. The cytotoxic T lymphocytes elicited by such an exposure are re-

Table 6–8. Comparison of class I and class II antigens.

	Class I	Class II
Antigens included	HLA-A, -B, -C.	HLA-D, -DR, -DQ, -DP.
Detection	Serologic.	HLA-DR, -DQ—serologic; HLA-D—MLR; -DP—PLT.
Tissue distribution	Wide—virtually on every cell.	Restricted to immunocompetent cells, particularly B cells, and macrophages.
Functions	Target of CML; recognized during graft rejection. Restrict CML of virus-infected cells.	MLR, PLT; important for antigen presentation, effective interaction between immunocompetent cells.

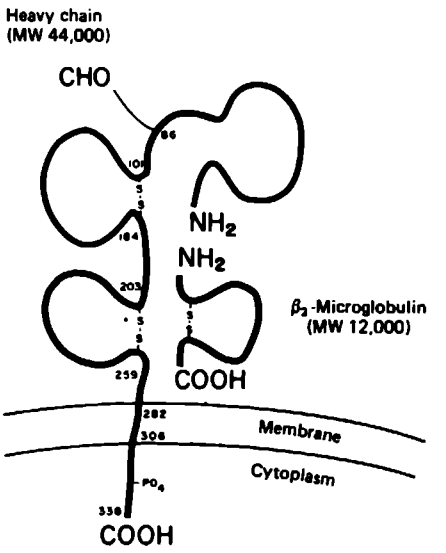

Figure 6–6. A schematic representation of an HLA class I molecule. The molecule consists of a 44,000-MW glycoprotein, the heavy chain, which bears the antigenic determinant, in a noncovalent association with a 12,000-MW nonpolymorphic protein, β_2-microglobulin. NH_2 = amino terminus; COOH = carboxyl terminus. CHO = carbohydrate side chain; –S–S– = disulfide bond. PO_4 = phosphate. Numbers indicate amino acid residues where certain features are found.

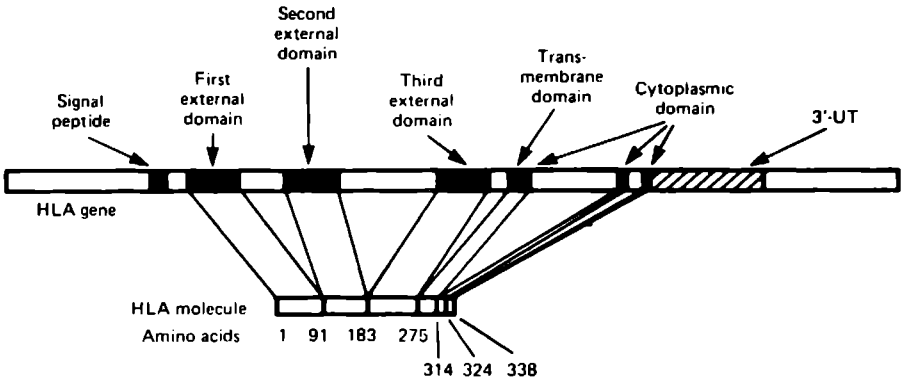

Figure 6–7. Schematic representation of an HLA class I genomic gene. The organization of the exons and introns, and the corresponding protein are shown. 3'-UT = 3'-untranslated region.

stricted in their killing to those target cells which bear *both* the same viral (or minor histocompatibility) antigen *and* the same class I antigen as were present on the sensitizing cell. These T lymphocytes will not kill target cells bearing the same viral (or minor histocompatibility) antigen and a different class I antigen, nor will they kill cells bearing the correct class I antigen and a different viral (or minor histocompatibility) antigen. Class I HLA antigens have been shown to restrict the killing of H-Y (male) antigen-bearing cells by autologous female cells and the killing of influenza virus-infected cells. Interestingly, the unexpected finding that a particular influenza virus-infected HLA-A2 target cell was not killed by an appropriate killer T lymphocyte led to the discovery that this HLA-A2 antigen, though serologically indistinguishable from other HLA-A2 antigens, was a mutant with an identifiable structural difference.

B. Class II Antigens: Class II antigens are found chiefly on the surfaces of immunocompetent cells, including macrophages/monocytes, resting T lymphocytes (in low amounts), activated T lymphocytes, and particularly B lymphocytes (Table 6–8). The HLA-DR and -DQ antigens are serologically detectable alloantigens. DP antigens were originally defined by primed lymphocyte typing, but now at least some DP antigens can also be detected by monoclonal antibodies. HLA-D antigens are the stimulator antigens of the MLR, and it is this reaction that is used to define and detect these antigens (see above). The finding that anti-HLA-DR antisera could inhibit the MLR was interpreted as indicating that the HLA-D and -DR antigens were at least in close proximity on the cell surface. However, it is most likely that the anti-HLA-DR antisera used in these blocking studies were in actuality detecting a number of different class II antigens. Recent evidence suggests that although the major determinants inducing the MLR probably reside on the HLA-DR molecule (Table 6–9), class II antigens other than HLA-DR can also induce an MLR. It is therefore possible that what has been termed HLA-D in reality refers to lymphocyte-activating determinants on several different class II antigens and may not be a distinct entity. Because HLA-D antigens are not defined by antibodies and therefore cannot be isolated, nothing is known about their structure. Each of the remainder of the class II antigens is borne on a 2-chain molecule that consists of 2 glycoproteins of about MW 34,000 (α chain) and about MW 29,000 (β chain) in noncovalent association (Fig 6–8).

The detailed structure of an HLA-DR class II molecule has recently been elucidated. Complete amino acid sequences of the HLA-DR α and β chains were derived from recombinant DNA nucleotide sequences or from conventional protein sequencing (or both). The α chain and β chain are composed of 229 and 237 amino acids, respectively (Fig 6–8). Like the HLA class I heavy chain, the α and β chains each consist of 3 regions—an extracellular hydrophilic region, a transmembrane hydrophobic region, and an intracellular hydrophilic region, the last 2 of which anchor the chains in the cell membrane. The α chain intracellular hydrophilic region can be phosphorylated. The α chain extracellular hydrophilic region contains 2 extracellular domains (residues 1–84 and 85–178), each of which has an attachment site for oligosaccharide and the second of which contains an intrachain disulfide bond. The β chain extracellular hydrophilic region also contains 2 extracellular domains (residues 1–91 and 92–192) the first of which bears oligosaccha-

Table 6–9. DR and Dw associations.

HLA-DR Antigen	Associated HLA-Dw Antigen(s)
DR1	Dw1
DR2	Dw2, Dw12
DR3	Dw3
DR4	Dw4, Dw10, Dw13, Dw14, Dw15
DRw11(5)	Dw5
DRw13(w6)	Dw6, Dw18, Dw19
DRw14(w6)	Dw9, Dw16
DR7	Dw7, Dw11, Dw17
DRw8	Dw8

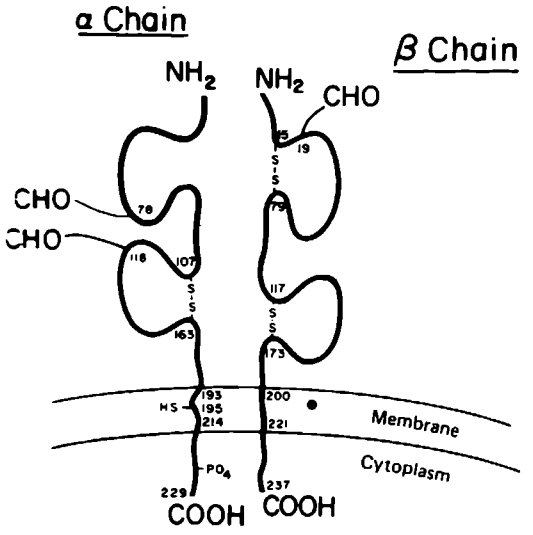

Figure 6–8. Schematic represention of an HLA-DR molecule. The molecule consists of a 34,000-MW glycoprotein (the α chain) in a noncovalent association with a 29,000-MW glycoprotein (the β chain). (Abbreviations and numbers as in Fig 6–6.)

side and both of which contain intrachain disulfide bonds. The second extracellular domain of both the α and β chains shows significant homology to immunoglobulin constant region domains. Comparative studies employing 2-dimensional gel electrophoresis, tryptic peptide mapping, and most recently amino acid sequencing have indicated that between molecules bearing different HLA-DR antigens, the α chains appear extremely similar, whereas the β chains are highly variable. This finding has suggested that the HLA-DR antigenic determinant resides on the β chain.

The structural features of the DQ molecule are similar to those of DR. The DQ α and β chains have 234 and 229 amino acids, respectively. In contrast to the DR molecules, *both* the DQ α and β chains are highly variable. Data indicate that the structural features of the DP molecules parallel those of the DR and DQ molecules.

The amino acid sequence data have also allowed homologies to be discerned between human and mouse class II antigens. These sequences indicate that the molecules bearing the HLA-DR alloantigens are homologous to the mouse molecules bearing the I-E Ia antigens and that the molecules bearing the DQ alloantigens are homologous to the mouse molecules bearing the I-A Ia antigens.

Recent information resulting from the application of molecular biologic techniques to the study of class II genes has indicated that there are several α and β genes within each subregion (Fig 6–9). The DR subregion contains one α chain gene and up to three β chain genes, although one of the latter may be a pseudogene (ie, it does not determine a product). The DR α chain can combine with any of the DR β chains to produce a DR molecule. The DQ and DP subregions each contain two α chain genes and two β chain genes. To date, there has been no example where an α chain determined by one subregion associates with a β chain determined by a different subregion.

The structures of the genes determining the DR α and DQ β chains are shown in Fig 6–10. Like the class I genes, they are composed of exons and introns, with the exons roughly corresponding to the protein domains. Other class II genes have similar structures. For the DR, DQ, and DP antigens, the genes determining both the α and β chains have been definitively mapped within the HLA complex.

The HLA-D antigens were discovered in 1972 owing to their ability to elicit an MLR. They are also thought to be the antigens principally responsible for the in vivo correlate of the MLR, the graft-versus-host reaction. As noted above, the lymphocyte-activating determinants are located on several different class II molecules. Presumably through this role, the class II antigens have been implicated in the sensitization phase (ie, the afferent limb) of cell-mediated cytolysis. This is in contrast to the effector phase (efferent limb), where the HLA class I molecules are important as target molecules.

The previously mentioned function relates to the artificial situation in which cells bearing different HLA antigens have been mixed. In the physiologic situation where cells bearing identical HLA antigens are required to interact productively, the HLA-DR as well as other class II antigens have been shown to be involved in antigen presentation by macrophages to T lymphocytes and in efficient collaboration between immunocompetent cells. Human suppressor factors that suppress an MLR have been shown to react with anti-HLA-DR alloantisera. In addition, some factors

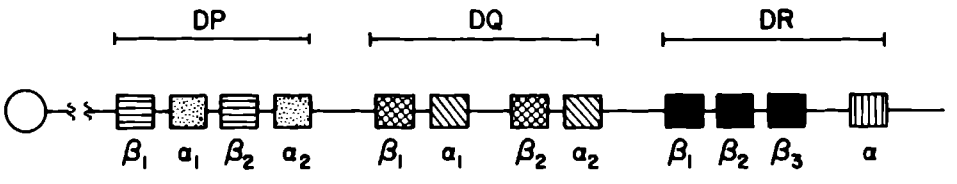

Figure 6–9. Schematic representation of the HLA class II region. The DP, DQ, and DR subregions and their constituent α and β chain genes are shown.

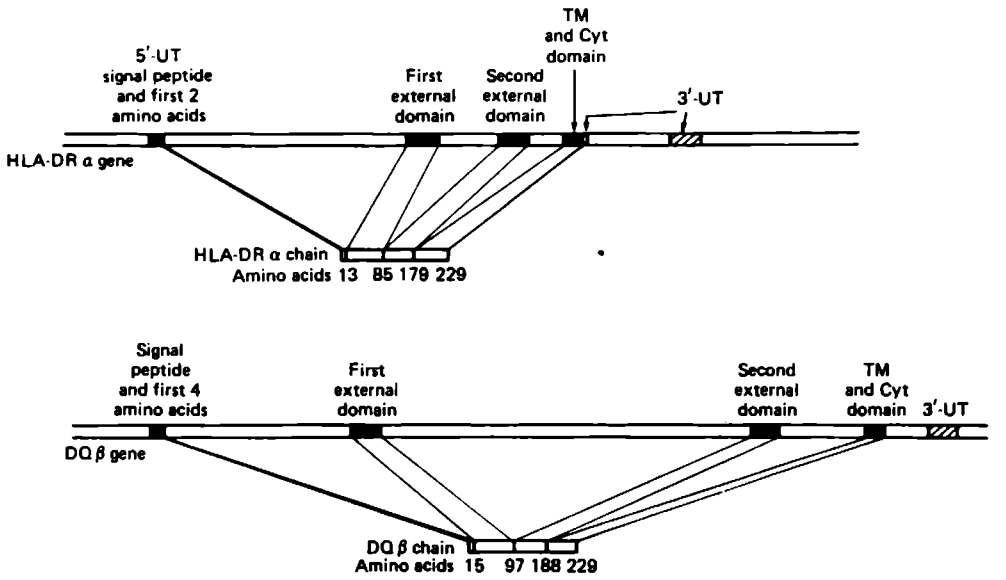

Figure 6–10. Schematic representation of HLA class II genomic genes. The organization of the exons and introns of an HLA-DR α gene and a DQ β gene and the corresponding proteins are shown. 5'-UT = 5'-untranslated region; TM = transmembrane region; Cyt domain = cytoplasmic domain; 3'-UT = 3'-untranslated region.

will suppress the MLR response of T cells only if both the factor and the T cell are reactive with the same anti-HLA-DR antiserum.

The DP antigens have been shown to be distinct from all other class II antigens. They can elicit a strong secondary proliferative response and can act as target antigens of cytotoxic T lymphocytes. Their function is presumed (not proved) to be similar to that of other class II antigens.

IMMUNE RESPONSE GENES

The search for human immune response (Ir) genes has been stimulated by the recognition of the overall homology of the HLA complex with animal major histocompatibility complexes and by the finding that the animal homologs of the region determining the class II antigens are the mapping sites for immune response genes. There is now some evidence that human Ir genes do exist. In humans, allergy to ragweed antigen Ra5 has been found to be highly associated with HLA-DR2 and is almost certainly a manifestation of a human Ir gene. Hyperresponsiveness to collagen has been shown to be associated with HLA-DR4 and appears to be secondary to a lack of collagen-specific suppressor T cells. Low responsiveness to cedar pollen, to schistosomal antigen, and to streptococcal cell wall antigen has been linked to the HLA complex, shown to be dominant, and interpreted as evidence for immune suppressor genes. Possible additional evidence for the presence of Ir genes comes from the HLA-linked disease susceptibility genes and HLA-disease associations discussed below.

HLA & DISEASE

Diseases associated with HLA antigens have several characteristics that should be noted. In general, these diseases (1) are of unknown cause and unknown pathophysiologic mechanism, with a hereditary pattern of distribution but weak penetrance; (2) are associated with immunologic abnormalities; and (3) have little or no effect on reproduction.

Both population and family studies have been used to demonstrate the relationship between marker genes within the HLA complex and various disease states. The 2 types of studies yield different types of information. Population studies permit a statistically significant association between a particular HLA marker gene and a particular disease. Such associations cannot be interpreted as proof of genetic linkage between a **disease susceptibility gene** and the HLA marker gene, since association does not necessarily indicate genetic linkage nor does linkage necessarily indicate association. For example, if a disease susceptibility gene was on a chromosome other than chromosome 6 and therefore not linked to HLA, but the presence of a particular HLA antigen was necessary for the phenotypic expression of that disease susceptibility gene, then an HLA-disease association would be established. Conversely, a gene such as phosphoglucomutase 3 (PGM_3) is on chromosome 6 and therefore linked to HLA, but no HLA-specific association with PGM_3 is found. In contrast, family

studies can demonstrate linkage between a disease susceptibility gene and the HLA marker. Because population studies are easier to perform, most of the data on HLA and disease derive from this type of study.

The association of a particular disease with a particular HLA antigen is quantitated by calculating the "relative risk." The relative risk (RR) can be stated as the chance an individual with the disease-associated HLA antigen has of developing the disease compared to an individual who lacks that antigen. It is calculated by the following formula:

$$RR = \frac{p^+c^-}{p^-c^+}$$

where p^+ = the number of patients possessing the particular HLA antigen,
c^- = the number of controls lacking the particular HLA antigen,
p^- = the number of patients lacking the particular HLA antigen, and
c^+ = the number of controls possessing the particular HLA antigen.

The higher the relative risk (above 1), the more frequent is the antigen among the patient population.

In contrast, the absolute risk (AR) is the chance an individual who possesses the disease-associated HLA has of actually developing the disease. It is calculated by the following formula:

$$AR = \frac{p^+}{c^+} \times P$$

where p^+ and c^+ are as above for the relative risk, and
P = prevalence of the disease in the general population.

The prototype of HLA-disease associations, that of **ankylosing spondylitis** with HLA-B27, can be used to illustrate these concepts. Ninety percent of American Caucasian patients with ankylosing spondylitis possess HLA-B27, compared to approximately 9% of American Caucasian controls. The relative risk is therefore $p^+c^- \div p^-c^+ = (90 \times 91) \div (10 \times 9) = 91$. Thus, an HLA-B27-positive individual has 91 times the risk of an HLA-B27-negative individual of developing the disease. The prevalence of clinically apparent severe ankylosing spondylitis is approximately 0.4%. The absolute risk is calculated as $90 \div 9 \times 0.004 = 0.04$. Therefore, of 100 HLA-B27-positive individuals, only 4 will actually develop clinically severe ankylosing spondylitis.

Because there is usually a significant difference in the frequency of a given antigen between different racial groups, it is always necessary to compare a patient group with a control population of the same race. Thus, for example, HLA-B27 is found in 48% of American black patients with ankylosing spondylitis, compared to 2% of American black controls, yielding a relative risk of 37.

In some cases, a disease may be associated with antigens determined by 2 different HLA loci. The actual association is frequently only with one antigen, but an apparent association with the second antigen is seen because of the phenomenon of linkage disequilibrium between the genes determining the 2 antigens (see above). The actual or primary association can usually be ascertained by statistically testing each antigen for disease association with the influence of the second antigen removed.

Various diseases have been associated with antigens determined by almost all HLA loci. Thus, for example, idiopathic hemochromatosis has been associated with HLA-A3, ankylosing spondylitis with HLA-B27, rheumatoid arthritis with HLA-DR4, Sjögren's syndrome with DRw52, and pauciarticular juvenile rheumatoid arthritis with complement haplotypes. A growing list of diseases has been associated with class II antigens, and this association has been interpreted by some to indicate that the class II antigens are marker antigens for closely linked immune response genes that somehow predispose to disease. An interesting finding in this regard is that several documented or presumed autoimmune diseases have been found to be associated with HLA-DR3 (Table 6–10). Most recently, specific class II region restriction endonuclease fragments have been associated with particular diseases. Space considerations preclude a listing of all HLA-disease associations.

Family studies provide an opportunity to establish definite linkage between a disease susceptibility gene and the HLA complex and may provide evidence for simple dominant or recessive inheritance. Thus, for example, a dominant disease susceptibility gene for ankylosing spondylitis with a penetrance of 0.38 has been found to be in very strong linkage disequilibrium with HLA-B27. Other HLA-linked disease susceptibility genes include those for idiopathic hemochromatosis, congenital adrenal hyperplasia, and insulin-dependent diabetes mellitus.

Several hypotheses have been advanced to explain HLA-disease associations. Three of these appear to be most likely. The first hypothesis holds that HLA antigens are merely markers for **immune response genes, or immune suppressive genes.** The homology of the region determining the human class II antigens with the animal I regions and the increasing evidence for Ir and Is genes in humans make this an attractive theory.

Table 6–10. Diseases of known or presumed autoimmunity associated with HLA-DR3.

Systemic lupus erythematosus
Sicca syndrome
Myasthenia gravis
Dermatitis herpetiformis
Insulin-dependent diabetes mellitus
Graves' disease
Idiopathic Addison's disease
Celiac disease
Autoimmune chromic active hepatitis

An implicit assumption of this hypothesis is that there is an agent responsible for a particular disease and that an Ir or Is gene regulates the ability to respond to the agent. The immunologic response (or lack thereof) to the agent may then predispose to disease. The finding that HLA-DR4 individuals (with or without rheumatoid arthritis) have an abnormally high response to collagen secondary to a lack of suppressor T cells for collagen lends credence to this postulate.

The second postulate suggests that HLA antigens may act as receptors for etiologic agents. If particular HLA antigens act as receptors for viruses, toxins, or other foreign substances and these substances are the etiologic agents for given diseases, then HLA-disease associations would result. The finding that HLA antigens bind Semliki Forest virus has been interpreted to support this postulate.

The third hypothesis is that of molecular mimicry. It postulates that the disease-associated HLA antigen is structurally and immunologically similar to the etiologic agent for the disease and further postulates one of 2 alternatives. The first alternative holds that because of the similarity between the etiologic agent and the HLA antigen, no immune response is mounted, and therefore the etiologic agent produces disease without any interference. The second alternative suggests that a vigorous immune response is mounted against the etiologic agent. Because of the similarity of the agent and the HLA antigen, the immune response is turned against the HLA antigen, and this "autoimmune" response then produces disease. The finding of cross-reactivity between certain microorganisms and certain HLA antigens supports this theory.

Other mechanisms besides those discussed above have also been suggested. It should be emphasized that different mechanisms may be operating in different HLA-disease associations and that more than one mechanism may be operating concurrently to produce disease. Further studies are necessary to clarify these issues.

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Lymphocytes

7

I. T CELLS

John D. Stobo, MD

The thymus-derived lymphocytes (T cells) mediate 2 general types of immunologic functions: effector and regulatory. **Effector functions** include reactivity such as delayed hypersensitivity, allograft rejection, tumor immunity, and graft-versus-host reactivity. These effector functions reflect 2 general properties of T lymphocytes: their ability to secrete proteins (termed lymphokines) and their ability to kill other cells (cytotoxicity). The **regulatory functions** of T cells are represented by their ability to amplify cell-mediated cytotoxicity by other T cells and immunoglobulin production by B cells. These functions also require synthesis of lymphokines.

An understanding of the function of T cells has been aided by the delineation of certain molecules expressed on their surface: The most important of these are listed in Table 7–1. Each of the listed antigens, detected with monoclonal antibodies, are expressed on a molecule or molecules that play an important role in T cell differentiation and function.

THE ANTIGEN RECEPTOR HETERODIMER

The results of studies in the late 1960s and early 1970s clearly demonstrated that T cells are antigen-specific. Therefore, it was clear that T cells must have a receptor capable of recognizing antigen. Only recently have the molecules comprising the receptor and the genes that code for them been characterized. The receptor consists of 2 chains, an acidic α chain (MW 45,000–55,000) and a more basic β chain (MW 40,000–50,000), which are linked by a disulfide bond on the T cell surface (Fig 7–1). Both α and β chains are integral membrane proteins each extending 4–5 amino acids into the cytoplasm.

Both chains can be divided into variable and constant region domains. The variable region interacts with antigen, and thus the amino acid sequences of the variable region of different antigen specific T cell clones are different. The amino acid sequences of the constant regions from these same clones are similar. This general structure is analogous to the variable and constant region domains noted for immunoglobulin molecules (see Chapter 5).

A more complete understanding of the structure of the antigen receptor and the genetic mechanisms that can lead to antigenic diversity among T cells comes from studies of the genes which encode the α and β chains (Fig 7–1). The genetic locus containing α chain genes is present on the 14th chromosome, while the genetic locus containing β chain genes is on the seventh human chromosome. These genetic loci can be further divided into the following regions: **variable region genes (V), diversity segment genes (D), joining region genes (J),** and **constant region genes (C).** The V, D, and J regions represent gene clusters and not a single gene. For example, there are approximately 60 different V region genes for the α chain and 21 different V region genes for the β chain. Although there do not appear to be any D region genes for the α chain, there are at least 2 for the β chain. There are 40 J region genes for the α chain, and 12 J region genes

Table 7–1. Molecules on the T cell surface.

Molecule*	Molecular Weight	Percent Positive		Comments
		Thymocyte	Peripheral T Cell	
CD2 (T11)	50,000	95	100	SRBC receptor
CD3 (T3)	19,000 22,000 25,000	90	100	3 molecules associated with antigen receptor (Ti)
CD4 (T4)	62,000	80	65	Involved in recognizing class II molecules
CD8 (T8)	76,000	80	35	Involved in recognizing class I molecules
CD25	55,000	...	...	Receptor for IL-2
T	45,000 55,000	85	100	Heterodimer that recognizes antigen plus MHC gene products

*CD = cluster of differentiation. (Old terminology in parentheses.)

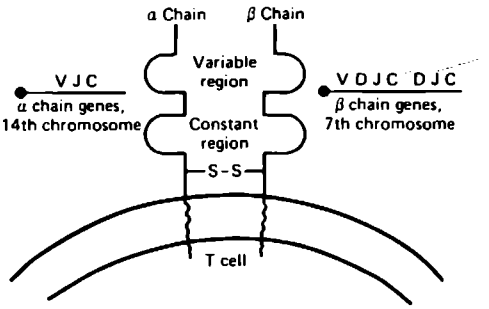

Figure 7–1. The T cell antigen receptor is a disulfide-linked heterodimer consisting of an α and a β chain. The genetic locus encoding for the α and β chains is on the 14th and seventh human chromosomes. Each locus is made up of families of genes called variable (V), diversity (D), joining (J), and constant (C).

for the β chain. There is one C region gene for the α chain, and there are two C region genes for the β chain. Rearrangements occur among these genes in a fashion analogous to that demonstrated for immunoglobulin genes (see Chapter 5). This provides the diversity to account for the existence in each individual of more than 1 million distinct T cell clones, each of which has a different antigen specificity. Diversity can be accounted for by 3 major mechanisms. First, each different V region gene can code for an α or a β chain that has a different antigen specificity. Therefore, in the case of the α chain, 60 different antigen specificities are coded for by the 60 different V region genes; and in the case of the β chain, 21 different antigen specificities are coded for by the 21 different V genes. Second, junctional diversity is created when a single V region gene joins with a different D or J region gene. For example, joining of one V region and one D or J region gene will result in an α or a β chain with a particular antigen specificity. However, if the same V region gene combines with another D or J region gene, then the specificity can be for a different antigen. The molecular mechanisms that result in the joining of special V, D, and J region genes are similar to those which account for the joining of immunoglobulin V, D, and J region genes (see Chapter 5). Third, it appears that the antigen specificity of the α/β chain heterodimer is due not only to the primary structure of the α or the β chain. Instead, antigen recognition is determined by interactions between the α and the β chain. Therefore, different antigen specificities of the receptor are created when a single α chain interacts with different β chains. This is analogous to the situation noted for immunoglobulins in which antigen specificity is inherent in a conformational determinant generated by interactions between the light and heavy chains.

Another genetic locus, termed the γ locus—which has V, J, and C regions and which gives rise to a mature mRNA in T cells—has been described. Although the gene is present in all cells, rearrangement of the gene and the presence of mRNA have been detected only in T cells, particularly cytotoxic T cells. No protein product for the mRNA has been detected inside or on the surface of T cells. Whether or not this genetic locus (termed the γ genes) plays any role in T cell recognition of antigen remains to be determined.

T CELL RECOGNITION OF ANTIGEN

It is clear that the α/β heterodimer represents the T cell antigen receptor. It is equally clear that the receptor does not recognize soluble antigen alone. It has been very difficult to consistently demonstrate binding of antigen to the surface of T cell clones that have specificity for that antigen. Instead, the antigen receptor recognizes antigen in conjunction with products of MHC genes (ie, class I and class II molecules; see Chapter 6). In the case of soluble antigens, recognition occurs in conjunction with class II molecules, whereas for viral antigens, recognition is in conjunction with class I molecules. Moreover, for large, soluble antigens, the antigen must be processed by an appropriate accessory cell such as a macrophage or dendritic cell. The sequence of events involved in T cell recognition of antigen proceeds as follows. The antigen is phagocytized by an antigen-presenting cell, internalized, processed, and then expressed on the cell surface in conjunction with class I or II MHC molecules. The T cell antigen receptor heterodimer then recognizes the antigen plus the MHC gene product (Fig 7–2). Recognition of antigen alone or MHC gene product alone is not sufficient to signal T cell activation. Only the complex can be appropriately recognized by the T cell antigen receptor heterodimer.

This sequence of events raises many questions. It is known that antigen processing is an energy-dependent process which results in degradation of the antigen. It

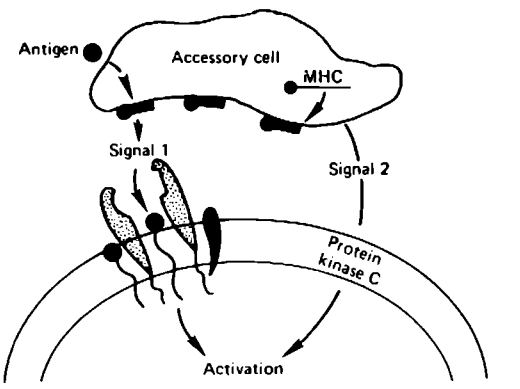

Figure 7–2. At least 2 signals are required for T cell activation. One is transmitted through the antigen receptor/CD3 complex and is represented by antigen seen in conjunction with MHC gene products. The second is represented by materials that can activate protein kinase C.

is not known just where in the antigen-presenting cell this occurs or what mediates the degradative process. It has been hypothesized that there is a physical interaction between antigen and class I or II MHC molecules. These MHC molecules have been postulated to represent the accessory cell's receptor for antigen. However, with only one or 2 exceptions, it has not been possible to demonstrate a physical association between antigen alone and class I or II MHC molecules. However, in the presence of a T cell clone specific for the antigen under study, the strength of the bond between the antigen and MHC gene products is markedly increased, and binding of the antigen to the MHC determinant can be detected. Therefore, it appears that the T cell stabilizes the association between the antigen and the surface MHC gene product. Moreover, it is the combination of antigen plus MHC gene product that is best recognized by the T cell antigen receptor.

The fact that the antigen receptor is made up of 2 molecules (α and β chains) and that the receptor recognizes 2 structures (antigen plus MHC molecules) suggests that one chain of the receptor might recognize antigen and the other MHC. In an attempt to confirm this, the structure of the α and β chains from different T cell lines that recognize the same antigen in conjunction with different MHC molecules has been compared. Preliminary results indicate that the structure of the α and β chains from each of the lines is different, suggesting that one chain does not simply recognize antigen and the other chain MHC or vice versa. Instead, a binding site for an associative determinant represented by antigen plus MHC is generated by interaction between the α and β chains. If this proves to be correct, it would represent another similarity between the T cell antigen receptor and antibody molecules. Although some exceptions exist, it is the combination of light and heavy chains that binds best to antigen when compared to the binding of isolated light and heavy chains.

THE CD3 COMPLEX OF MOLECULES

Three other molecules, termed CD3, are physically associated with the antigen receptor heterodimer on the T cell surface (Fig 7–2). The physical association between the antigen receptor heterodimer and CD3 is supported by several observations. For example, immunoprecipitates obtained from solubilized T cells using monoclonal antibodies against the antigen receptor heterodimer also contain each of the three CD3 molecules. Conversely, immunoprecipitation of the CD3 complex also co-precipitates the antigen receptor heterodimer. Removal of the antigen receptor heterodimer from the cell surface with monoclonal antibodies under capping conditions causes co-capping of the CD3 complex.

The three CD3 peptides have been termed CD3/γ 25,000-MW glycoprotein, CD3/δ (20,000-MW glycoprotein) and CD3/ξ (20,000-MW protein). Each of the CD3 molecules is an integral part of the T cell membrane and extends into the cytoplasm farther than either the α or β chain of the antigen receptor.

While several lines of evidence suggest that the T cell antigen receptor is a 5-chain complex consisting of the α and β chains of the receptor heterodimer and the γ, δ, and ξ chains of CD3, the role that each molecule or set of molecules plays in T cell activation is not clear. Examination of the amino acid sequence of CD3 molecules from T cell lines having different antigen specificities demonstrates them to be identical. Therefore, it is unlikely that the CD3 set of molecules plays a role in recognizing either antigen or MHC gene products. It is possible that the CD3 set of molecules functions to stabilize the antigen receptor so that a correct conformation of the receptor can be maintained. Alternatively, it is possible that while antigen recognition is the property of the antigen receptor heterodimer, transduction of the activating signal through the cell membrane to the interior of the cell is the property of CD3.

THE CD4 & CD8 MOLECULES

The CD4 and CD8 molecules also play an important role in T cell activation. Both the CD4 and CD8 molecules, as well as the genes that code for them, have been isolated. The study of these molecules and their genes indicates no polymorphism among T cells having different antigen specificity. No rearrangements occur in the CD4 or CD8 genes, and the CD4 molecules isolated from several different antigen-specific T cells demonstrate identical structure. The same situation applies to CD8 molecules isolated from different antigen-specific T cell lines. Therefore, the CD4 and CD8 molecules do not participate in antigen recognition.

The CD4 and CD8 molecules do play a role in the recognition of MHC gene products. The presence of CD4 on T cells indicates that the T cell is programmed to recognize class II molecules as part of its specificity, while T cells bearing CD8 are programmed to recognize class I molecules as part of their specificity (Fig 7–3). Antibodies with specificity for either CD4 or CD8 can block T cell activation, particularly among T cells seeing antigen for the first time, ie, unprimed T cells. Once a T cell has been primed, it appears that the CD4 and CD8 molecules are less important in their subsequent activation. These observations have led to the following hypothesis. The CD4 and CD8 molecules are not involved in signaling activation but instead represent a receptor that can increase interactions between the T cell and its target. The CD4 and CD8 molecules provide extra "glue" to increase interactions so that activation can occur. For primed cells, this extra "glue" is not as necessary, presumably because there has been some change in the antigen receptor heterodimer that enables it to mediate sufficient cell-cell interaction alone. Although class I and II MHC molecules have a variable region that differs

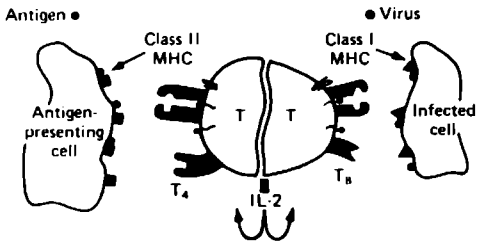

Figure 7–3. The presence of CD4 or CD8 molecules indicates whether the T cell is restricted to recognizing class II or class I MHC molecules. The CD4 and CD8 molecules may interact with the constant regions of the respective MHC molecules and increase cell-to-cell interactions.

among genetically distinct individuals and thus accounts for their serving as transplantation antigens, they also have a constant region which is much less polymorphic. Since the structure of CD4 and CD8 isolated from T cell lines restricted to recognize antigen in conjunction with different MHC molecules is identical, it is thought that the CD4 and CD8 molecules recognize the constant region of the MHC molecule.

THE CD2 MOLECULE

The CD2 molecule is the receptor by which the T cell forms rosettes with sheep red blood cells. The role CD2 plays in T cell function is not clear. Recent studies suggest that it may play a role in an alternative pathway of activation distinct from that mediated through the antigen receptor heterodimer. Monoclonal antibodies can detect 3 distinct epitopes in the CD2 molecule: $CD2_1$, $CD2_2$, and $CD2_3$. $CD2_1$ and $CD2_2$ epitopes are present on nearly all thymocytes and all resting peripheral blood T cells. $CD2_3$ appears only when T cells are activated. Addition of anti-$CD2_2$ to T cells induces expression of the $CD2_3$ epitope. Simultaneous addition of monoclonal antibodies against the $CD2_2$ and the $CD2_3$ epitopes induces T cell activation. Recently, a soluble material has been isolated from T cells that binds to the CD2 molecule on resting T cells and induces their activation. This activation occurs independently of any exposure of the cells to antigen and may represent a mechanism by which T cells responding to a specific antigenic stimulus can recruit other T cells in an antigenic nonspecific fashion.

T CELL ONTOGENY

The appearance of mature, functional T cells in the peripheral lymphoid tissue represents the culmination of a series of differentiation steps occurring in the thymus. Stem cells migrate to the thymus and then move from the cortex to the medulla and out into the periphery, a journey that takes 3 days. During intrathymic development, thymocytes acquire functional maturity as shown by changes in their cell surface phenotype as reflected by the expression of the cell surface molecules listed in Table 7–1 (Fig 7–4). Stem cells entering the thymus fail to express the CD2, CD3, CD4, or CD8 determinant. The earliest stage of differentiation in the cortex is manifested by the display of the CD2 molecule. At this point, the cell still fails to express the CD3, CD4, and CD8 molecules. Further differentiation in the cortex is represented first by the expression of CD3 and subsequently by the expression of both CD4 and CD8 on the same T cell. Within the thymic medulla, the T cell lineage splits into 2 parallel lines, each displaying CD2 and CD3 molecules. However, one population of thymic medullary cells is $CD4^+$ and $CD8^-$, while the other is $CD4^-$ and $CD8^+$. These 2 cell lineages then enter the periphery as 2 distinct T cell lines. The $CD2^+$, $CD3^+$, and $CD4^+$ cells represent 65% of peripheral T cells, and the $CD2^+$, $CD3^+$, and $CD8^+$ population represent 35% of peripheral blood T cells.

Two important related events occur during thymocyte differentiation. First, approximately 90% of the thymocytes undergo interthymic death and never emigrate into the periphery. Second, T cells learn to recognize self-MHC gene products. As discussed above, recognition of antigen by peripheral T cells requires that they see antigen in conjunction with MHC gene products. This recognition process is learned in the thymus. This may be best explained by the following example. Activation of virus-specific cytotoxic T cells requires the T cell to view the virus in conjunction with class I MHC molecules displayed by the virus-infected cell. If animals of strain *x* are infected with virus *y*, the cytotoxic T cells that are generated in the periphery will demonstrate specificity for *y* molecules seen in conjunction with *x* class I MHC molecules. If, however, the thymus of strain *x* is removed and replaced with a thymus from strain *z* animals, virus-specific cytotoxic cells appearing in the peripheral lymphoid tissue will have specificity for the same virus *y* that is recognized only in conjunction with strain *z* class I MHC molecules and not strain *x* class I MHC molecules.

The exact mechanism by which this self-recognition occurs, without the development of auto-aggressive T cells capable of recognizing self class I or II

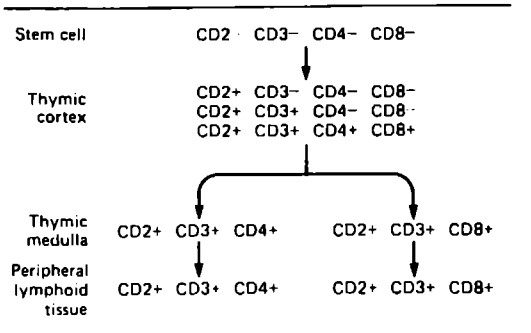

Figure 7–4. The CD phenotype of thymocytes and peripheral T cells.

MHC molecules themselves, is not clear. Stromal cells in the thymus clearly display class I MHC molecules and have also been reported to display class II MHC molecules. Direct binding of thymocytes to thymic stromal cells can be detected. During their differentiation within the thymus, T cells capable of recognizing the self class I or II molecules alone may be deleted and represent the 90% of thymocytes that die. In contrast, the 10% of T cells that react with the same molecules only in conjunction with antigen proceed to the periphery.

During their development in the thymus, genes coding for the antigen receptor undergo rearrangement, and the antigen receptor heterodimer becomes expressed. Using cDNA probes for the α and β chain genes as well as γ genes (this γ refers to the genes rearranged in T cells and not the γ chain of the CD3), it can be demonstrated that γ chains are rearranged first, next α chain genes, and finally β chain genes. Only when all 3 families of genes are arranged does the α/β heterodimer appear on the cell surface. As indicated, protein products of γ chains have not yet been identified in the T cell cytoplasm or on the cell surface. The expression of the α/β heterodimer coincides with the expression of CD3. This underscores the unique physical association between CD3 and the antigen receptor. By the time thymocytes emigrate to the periphery, their antigen receptors are expressed, and thus their antigen specificity is determined.

Immunocompetent T cells leave the thymus through the walls of postcapillary venules in the medulla and enter the bloodstream. They are subsequently distributed among the peripheral lymphoid tissues. Once there, the T cells localize in the thymus-dependent regions (the inner cortex) of lymph nodes, periarterial sheaths of the spleen, or intranodular areas in Peyer's patches. In less than 24 hours, the T lymphocytes leave by efferent lymphatics, move into the larger lymphatics and thence to the thoracic duct, and return into the bloodstream. The movement of lymphocytes from the circulation into lymphoid organs occurs through specialized areas in the blood vessels called postcapillary high endothelial venules. The lymphocytes bind to these venules by means of specific receptors, and there is a strong preference for binding to venules of those lymphoid organs from which the lymphocytes were originally isolated. For instance, if peripheral lymph node cells are injected back into an animal from which they were removed, they preferentially localize to the peripheral lymph nodes—not to the spleen or Peyer's patches. Identical preferential trafficking of Peyer's patches lymphocyte can be demonstrated. Evidence that the homing pattern of lymphocytes is due to binding to cell surface recognition units comes from studies in which lymphocytes are treated with enzymes that digest cell surface molecules. The lymphocytes so treated lose their homing pattern, and their distribution becomes random. An example of this preferential trafficking is the localization of lymphocytes involved in IgA antibody production to gut-associated lymphatic tissues.

The compartmentalization of lymphatic tissue (such as paracortical areas traversed by T cells, sinusoids containing macrophages and a reticular network of dendritic cells) seems to be well designed to facilitate the interaction between the various types of cells involved in the generation of an immune response.

BIOCHEMICAL EVENTS IN T CELL ACTIVATION

Studies of the biochemical events involved in T cell activation have been aided by 2 discoveries. First, lines or clones of T cells can be established using the T cell growth factor, **interleukin-2 (IL-2).** This allows the generation of large numbers of cells for study. Second, monoclonal antibodies with specificity for the antigen receptor heterodimer can substitute for antigen plus MHC as a signal for activation. Thus, one can use these antibodies to induce activation instead of searching for the antigen recognized by the T cell line under study.

Events known to be required for T cell activation can be summarized by the following (Fig 7–5; see also Chapter 15). Interaction between the antigen receptor/CD3 complex on the T cell and antigen plus MHC gene products on accessory cells generates 2 biologically active metabolites from membrane inositol. The first is inositol trisphosphate, which increases cytoplasmic free calcium by mobilizing calcium from bound intracellular stores in the endoplasmic reticulum. The second is diacylglycerol, which activates the enzyme protein kinase C. However, increases in cyto-

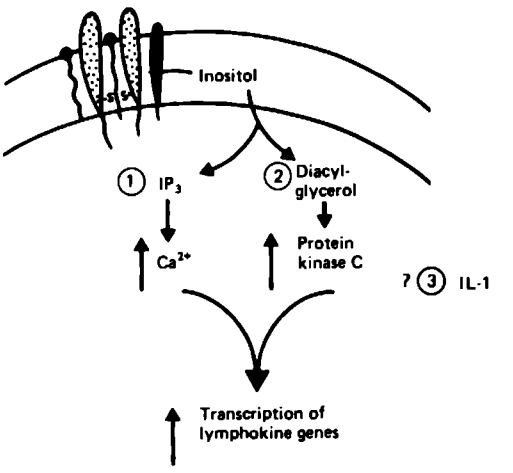

Figure 7–5. Stimuli required for T cell activation. Perturbation of the antigen receptor/T3 complex generates inositol triphosphate (IP_3) and diacylglycerol from inositol. IP_3 mobilizes calcium from bound intracellular stores, while diacylglycerol activates protein kinase C. IL-1 represents a third signal required for the activation of resting T cells. The metabolic events generated in IL-1 are unknown.

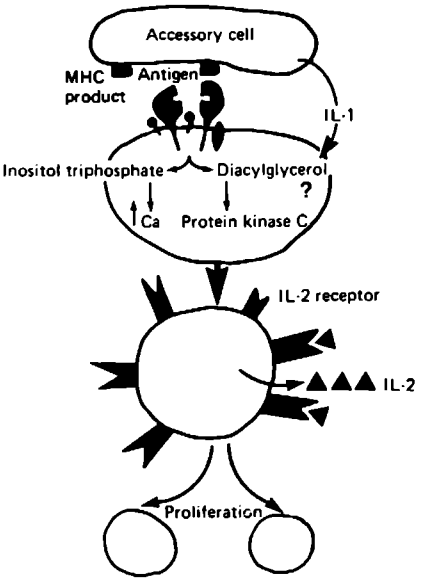

Figure 7–6. T cell proliferation. In the presence of 3 stimuli—increase in cytoplasmic free calcium and activation of protein kinase C and of IL-1—IL-2 and IL-2 receptor synthesis is initiated. Since IL-2 is a T cell growth factor, this leads to a brief burst of cell proliferation.

plasmic free calcium and activation of protein kinase C, while necessary, are not by themselves sufficient to activate resting T cells. Another, as yet undefined, signal is required. It is this signal which can be initiated by **interleukin-1 (IL-1).** Initial studies indicated that incubation of T cells with calcium ionophores, which increase cytoplasmic free calcium directly, plus phorbol esters, which activate protein kinase C, could activate resting T cells. Based on this observation, it was concluded that only 2 signals are sufficient to induce T cell activation. Subsequently, it was found that phorbol esters can transmit signals in addition to protein kinase C activation. It is this additional signal which can be mediated by IL-1.

In order for T cell activation (at least as measured by T cell proliferation) to proceed to completion, other events must occur. The first is the secretion of IL-2, and the second is enhanced expression of the receptors for this T cell growth factor (Fig 7–6). Synthesized and secreted IL-2 can feed-back on the cell synthesizing this factor and increase expression of IL-2 receptors initiated by T cell activation. Therefore, IL-2 acts as an autacoid as it expands that particular T cell clone. This accounts for the amnestic response following initial exposure to a specific antigen. Continuous exposure of the activated T cell to IL-2 results in down-regulation of the IL-2 receptor, so that continuous replication and the potential for malignant transformation do not occur. The biochemical and molecular events that link IL-2 and IL-2 receptor synthesis to the early events involved in T cell activation remain to be elucidated.

T CELL EFFECTOR FUNCTION

The number of T cells specific for a single antigenic determinant is exceedingly small (one out of every 100,000 T cells). Reactivity of T cells to a foreign antigen containing (for example) 10 different antigenic determinants would therefore not result in substantial immune reactivity unless there were some way by which T cells could enhance their activity. This is accomplished through liberation of a collection of soluble materials termed **lymphokines.** Once T cells are activated by the signals required for antigen-specific activation, they can release these lymphokines. These lymphokines then act in an antigen-nonspecific fashion on other populations of mononuclear cells irrespective of their antigen specificity. Some of these lymphokines and their functions are listed in Table 7–2. Two prototypes of effector T cell function are delayed hypersensitivity and cytotoxicity. Both will be discussed in order to emphasize the afferent and efferent events involved in T cell effector function.

Delayed Hypersensitivity

Delayed hypersensitivity is crucially involved in host defense against viruses, fungi, mycobacteria, and other organisms that replicate intracellularly. Accessory cells operate at both the afferent and efferent limbs of this reactivity. Activation of T cells specific for fungal antigens, for example, requires that they be appropriately processed and presented by MHC molecule-positive accessory cells in conjunction with the synthesis of soluble materials such as IL-1. Enhancement of the reactivity of the activated T cells occurs by synthesis and liberation of lymphokines such as **migration inhibition factor.** This factor inhibits the random migration of macrophages through tissues, thereby resulting in their accumulation around the area of T cell activation. Other lymphokines such as **gamma interferon** enhance the cytolytic activity of the accumulated macrophages. Other factors may be important in the generation of giant cells and other events involved in granuloma formation that can occur as a consequence of the T cell response to antigens. Delayed hypersensitivity reactions are "delayed" be-

Table 7–2. Lymphokines synthesized by T cells.

Lymphokine	Action
Migration inhibition factor	Inhibits the random migration of macrophages.
Leukocyte inhibition factor	Inhibits the random migration of neutrophils.
Macrophage activation factor	Enhances the cytolytic activity of macrophages.
Gamma interferon	Has the same activities as macrophage activation factor.
Colony-stimulating factor	Supports the growth and differentiation of monocytes.
Fibroblast activation factor	Stimulates proliferation of fibroblasts.
Interleukin-3	Is similar to colony-stimulating factor.

cause time is required for synthesis of lymphokines and for their action to become manifest.

Effector cells required to initiate reactions of delayed hypersensitivity are included among the $T4^+$ population of lymphocytes. It is clear that this reactivity can be modulated in a negative sense by active suppressive influences existing among the $T8^+$ T cells. The relationship between these 2 populations of cells that determines the net expression of delayed hypersensitivity is exemplified by what occurs in response to infection with *Mycobacterium leprae*. Infection with this organism can cause 2 polar types of disease. **Lepromatous leprosy** is a disseminated disease in which enormous numbers of viable organisms are in the skin. **Tuberculoid leprosy** is a relatively limited form of the disease in which few viable organisms are present. Patients with lepromatous leprosy can be demonstrated to lack, in vivo and in vitro, T cell reactivity to *M leprae*. Individuals with tuberculoid leprosy demonstrate high levels of T cell reactivity that enables them to effectively clear the organism. It is possible that in lepromatous leprosy, T cells reactive to *M leprae* do not exist. Alternatively, it is possible that potentially reactive T cells do exist but that their reactivity is suppressed. Several observations indicate that the latter is actually the case. First, in the lesions of lepromatous leprosy, $T8^+$ cells predominate; whereas in the lesions of tuberculoid leprosy, $T4^+$ cells predominate. Second, depletion of $T8^+$ cells from the peripheral blood of individuals with lepromatous leprosy results in the appearance of in vitro reactivity to *M leprae*. Third, addition of exogenous IL-2 to in vitro cultures of cells from patients with lepromatous leprosy can restore T cell reactivity to the organism. Therefore, it appears that apparent absence of T cell reactivity to *M leprae* in lepromatous leprosy represents a predominance of immunosuppression rather than an absence of reactive cells. Furthermore, this suppression may represent a situation in which the synthesis, secretion, or activity of IL-2 is interfered with.

Cytotoxicity

The immune response to foreign transplantation antigens (ie, **alloantigens**) displayed by a grafted tissue is characterized by the generation of T cells that are **cytotoxic for the allograft.** The appearance of these cells occurs in 2 stages. In the first, $T4^+$ T cells recognize the class II molecules displayed by the allograft as foreign and are activated (Fig 7–7). A portion of this activation is represented by proliferation, and it is this proliferation that marks the in vitro mixed lymphocyte reaction—an assay used to measure the foreignness of alloantigens (see Chapter 6). The second stage involves the generation of cytotoxic effector cells among the $T8^+$ population of T cells. IL-2 secretion by $T4^+$ cells is necessary for the full development of cytotoxic effector $T8^+$ T cells. These cytotoxic cells recognize class I MHC molecules displayed by the allograft. In other words, the full generation of cytotoxic effector cells requires interactions among 2 phenotypically distinct populations of T cells. In this interaction, $T4^+$ T cells help in the development of effector $T8^+$ T cells through the generation of IL-2 (Fig 7–7). There has recently been some evidence that other soluble factors in addition to IL-2 are required for the appearance of differentiated cytotoxic effector T cells.

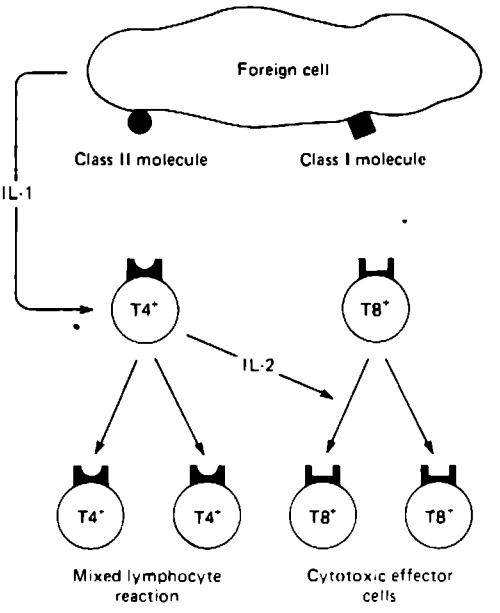

Figure 7–7. Events involved in the activation of T cells by alloantigens. In response to foreign class II molecules plus soluble materials such as IL-1, $T4^+$ cells proliferate and liberate IL-2. In conjunction with IL-2 and recognition of class I molecules, $T8^+$ cells can give rise to cytotoxic T cells. (Reproduced, with permission, from McCarthy DJ [editor]: *Arthritis and Allied Conditions*. Lea & Febiger, 1985.)

Cytotoxic T cells play an important role in the host defense against viruses. In order for the $T8^+$ population of cells to mediate cytotoxicity of virally infected cells, they must recognize viral determinants in conjunction with class I molecules displayed by the infected cells. Given that $T8^+$ T cells can recognize foreign class I molecules alone as well as viral determinants in conjunction with self class I molecules, an important question concerns the relationship between these 2 specificities and the T cell receptor recognizing each specificity. In order to determine this, clones of T cells cytotoxic for virus seen in conjunction with self class I molecules were developed and assayed for their cytotoxic activity against a panel of targets each of which displayed a different foreign class I molecule. The results of these studies demonstrated that the same clone of T cells capable of recognizing virus in conjunction with self class I molecules could recognize a single foreign class I specificity alone and that the receptor used for both reactivities is identical. Thus, it appears that reactivity to foreign class I molecules represents a cross-reaction mediated

by a T cell receptor that also has specificity for virus plus self class I.

A similar situation exists for the relationship between the receptor used to recognize antigen plus self class II molecules or foreign class II molecules alone. That is to say, the reactivity of $T4^+$ T cells to foreign class II molecules alone seems to represent a cross-reaction mediated by a receptor that can also see conventional antigen in conjunction with self class II MHC molecules.

The search for cytotoxic T cells led to the discovery of another cell that can kill a variety of tumor cells. These cells comprise a discrete population of large lymphocytes that can be distinguished by characteristic azurophilic granules in their cytoplasm. Based on their spectrum of lysis and morphology, they have been termed **natural killer (NK) cells** or **large granular lymphocytes (LGL).** Some NK or LGL are CD3-positive, suggesting that they are T cells. However, these cells do not appear to use the α or β chain of Ti as the receptor by which they recognize their target. Indeed, molecules used as their target receptor as well as ligands on the target cell recognized by the receptor are unknown. The lytic activity of NK cells can be markedly enhanced by gamma interferon. A material present in the cytoplasmic granules termed **granule cytolysin** may mediate lysis by insertion into the cell membrane of the target.

REGULATORY FUNCTION OF T CELLS

In addition to functioning as effector cells, T cells play a crucial role in regulating immunologic reactivities. The role $T8^+$ cells may play in the regulation of effector cells necessary for host defense against infection with *M leprae* has already been discussed. In addition to influencing reactivity of other T cells, regulatory populations of T cells play a crucial role in modulating the development of immunoglobulin-secreting plasma cells from immunoglobulin-bearing B cells. The $T4^+$ population of T cells contains those cells that **help B cells develop into immunoglobulin-secreting plasma cells,** while the $T8^+$ population of T cells contains cells capable of **inhibiting this differentiation.** It is also clear that the activation of $T8^+$ suppressor cells can be helped by cells existing within the $T4^+$ population. This help in the generation of suppression among a phenotypically distinct population of T cells is comparable in principle to the help necessary for the generation of $T8^+$ cytotoxic effector T cells.

Two pathways are involved in T cell help for immunoglobulin production: direct interactions between T cells and B cells and the secretion of soluble regulatory materials by the T cell. Direct contact between antigen-specific T cells and antigen-specific B cells may be necessary to focus or concentrate antigen in a manner sufficient to initiate B cell activation. This initial activation then results in the expression of certain receptors in the B cell surface capable of binding to growth and differentiation factors released by T cells. These growth and differentiation factors then can cause proliferation and expansion of specific clones of B cells as well as push B cell differentiation forward to the development of immunoglobulin-secreting plasma cells. At present, it appears that phenotypically distinct subpopulations of B cells exist. Whether or not direct interaction between helper T cells and B cells or simply the liberation of soluble materials by T cells is required for B cell differentiation depends on the subpopulation of B cells analyzed. It should be emphasized that T cells and B cells need not share the same antigenic specificity in order for T cells to provide help. The classic example of this is the interaction between T cells and B cells involved in the development of antibodies to haptens. **Haptens** are small antigenic molecules which by themselves cannot initiate antibody production. However, if a hapten is coupled to a larger molecule, termed a carrier, the hapten carrier conjugate can indeed induce the production of antibodies with specificity for the hapten. At a cellular level, this involves interactions between **carrier-specific T cells** and **hapten-specific B cells.** The carrier-specific T cells provide the help that allows the differentiation of the hapten-specific B cells into plasma cells producing antibody with specificity for the hapten.

Little is known about how suppressor cells exert their effect. Although a variety of suppressor materials have been described, they are for the most part poorly characterized. Therefore, it is difficult to know whether suppressor cells inhibit reactivity directly, by acting at the effector cell stage, or indirectly, by interfering with helper or amplifier influences necessary for full reactivity to appear. Interactions between T cells and B cells involved in B cell differentiation are discussed further below.

II. B CELLS

Daniel Levitt, MD, PhD, &
Max D. Cooper, MD

The existence of antibody molecules, or "antitoxins," has been recognized for over a century. However, the cellular source of immunoglobulins remained unclear until antibody production was correlated with the presence of plasma cells in lymphatic tissues. The antibody products of plasma cells were then directly visualized, using the Coons fluorescent antibody technique, at about the time when boys with congenital agammaglobulinemia were noted to have no germinal centers or plasma cells. The thymus was then discovered to be an essential source of small immunocompetent lymphocytes, and small lymphocytes in the circulation were shown to give rise to plasma cells in peripheral lymphoid tissues. Despite earlier studies relating the removal of the avian bursa of Fabricius with defective antibody responsiveness, it was believed until the mid 1960s that the thymus either

regulated or was the site of early development of the lymphoid precursors of antibody-forming cells. This concept was finally rejected upon demonstration that lymphoid development proceeds along 2 separate pathways, with the thymus-independent line of immunoglobulin-producing cells beginning its differentiation in the avian bursa. This lineage of cells was termed B cells, and owing to rapidly improving technology, much has since been learned about the life history of B cells and their role in immunoglobulin production.

MICROENVIRONMENT

The earliest site of development for blood cell precursors may be in the para-aortic mesenchyme of the embryo. These embryonic stem cells migrate into the yolk sac, where they form blood islands. Development of B lineage cells begins later than erythroid and myeloid cell differentiation and is confined to special microenvironments. In birds, B cells are generated in the hindgut lymphoid organ called the **bursa of Fabricius.** In mammals, cells of B lineage are initially generated in the fetal liver when hematopoietic stem cells migrate there from the yolk sac. This process begins during the eighth week of human gestation. The **fetal liver** continues to be a major site for production of erythroid, myeloid, and B cells until well into the second trimester. Stem cells then populate the bone marrow, which replaces the liver as the major hematopoietic tissue. Thereafter, B cells and other types of blood cells are continuously produced in bone marrow throughout life. It has been estimated that approximately 10^8 B cells are formed every day in the bone marrow of a mouse and many more in the bone marrow of a human. This is more than enough to generate the estimated 10^6–10^8 different clones of B cells, each producing an antibody of a different specificity.

Specific requirements for the induction of B cell differentiation in these hematopoietic microenvironments are still unknown. Fetal liver contains a population of nonadherent cells that appear to be important for the initial development of B lineage cells. In **adult bone marrow,** such inductive cells have the property of adhering to plastic surfaces. Multipurpose growth factors that can affect cells of both erythroid and myeloid lineages may also affect the growth and development of early B lineage cells. The existence of molecules that specifically stimulate B cell development, comparable to thymic hormones for T cells and erythropoietin for erythrocytes, also remains an unproved possibility.

PHENOTYPE & FUNCTION OF B CELLS AT DIFFERENT DEVELOPMENTAL STAGES

B cells develop from a pluripotential stem cell that can give rise to all of the different types of hematopoietic cells (Fig 7–8). As evidence of this in humans, in-

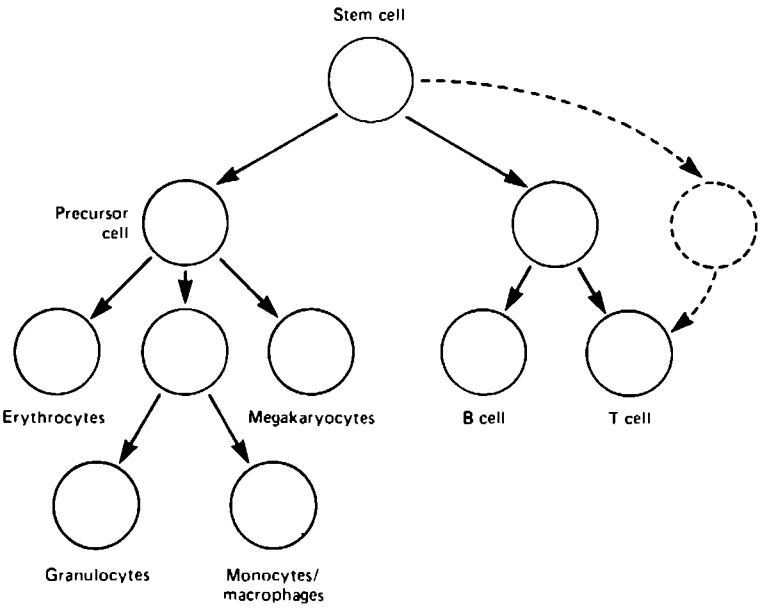

Figure 7–8. Pathways of hematopoiesis. Pathways by which the common stem cell develops into T cells, B cells, monocytes, and other cell lines are shown. The dotted lines indicate the potential pathway by which T cells develop from a precursor distinct from that leading to the development of B cells. Natural killer (NK) cells, not shown in the figure, are also derived from multipotent hemopoietic stem cells, perhaps via a common lymphoid precursor cell.

dividuals with chronic myelogenous leukemia and a marker Philadelphia chromosome in their malignant cells possess the same chromosomal abnormality in all hematopoietic cell lines, including both B and T lymphocytes.

During early development, apparently random inactivation by heterochromatinization of one of the pair of X chromosomes permits only a single allele to be expressed. Certain female heterozygotes exhibit only one isozyme in all types of blood cells, while the other tissues express isozyme patterns encoded for by both complementary alleles. Therefore, random inactivation of the X chromosome must occur prior to commitment of hematopoietic stem cells along any of the specific developmental pathways; these findings also suggest that a **common stem cell** serves as progenitor for all blood cell elements.

Individuals with polycythemia vera demonstrate aberrant development of erythrocytes, platelets, and myeloid cells but relatively normal lymphoid elements. This suggests early divergence of blood cell development along at least 2 pathways. In rodents, use of marker chromosomes or radiation-induced chromosome lesions has provided evidence for a common origin of lymphoid, myeloid, erythroid, and megakaryocytic cells, followed by an early separation of lineages along 2 basic paths. This early branching between lymphoid versus other hematopoietic precursor cell populations can be seen even more clearly when **colony-forming cells (units)** are grown **in culture (CFU-C)** or **in the spleens (CFU-S)** of irradiated mouse recipients. Such colonies may contain myeloid, erythroid, and megakaryocytic elements but lack lymphoid cells and their precursors. Thus, despite their common stem cell origin, T and B cells appear to diverge from other hematogenous elements (and from each other) early in their developmental history.

Pre-B Cells

Precursor B cells, generated first in the fetal liver and then in bone marrow, lack immunoglobulin products but may express other characteristic surface molecules that can be identified by specific monoclonal antibodies. These cells divide and undergo rapid transition to become large lymphoblasts with cytoplasmic μ **heavy chains** but no **light chains** (Fig 7–9). Such cells can be found in human fetuses during the eighth week of gestation.

In order to express μ chains, the precursor cells rearrange one of their many variable genes (**V exon**) next to **D** (diversity) and **J** (joining) gene segments. D-J joining may occur first, followed by V-D joining (see Chapter 3). Next, the rearranged V-D-J gene complex is transcribed along with the μ heavy chain constant region (C_μ) genes. The μ chain messenger RNA is then processed, and a complete μ protein can now be synthesized. Although immunoglobulin gene rearrangements may occur on both homologous chromosomes, productive rearrangement apparently occurs on only one chromosome. This prevents the production of 2 types of heavy chains, having different variable regions, by a single pre-B cell. Light chain genes still remain in their germ line configuration at this stage in differentiation.

The pre-B cell phenotype (cytoplasmic μ chain$^+$, light chain$^-$, surface Ig$^-$) can first be demonstrated in lymphoblasts actively engaged in DNA synthesis. When the light chain genes are subsequently rearranged and expressed in a progeny subpopulation of small pre-B cells, DNA synthesis has abated, and surface immunoglobulin appears. The percentage of pre-B cells that actually complete all of these developmental stages to become B lymphocytes is unknown. Some may fail to achieve productive rearrangements of either heavy or light chain genes or may abort prematurely for other reasons.

Human pre-B cells express **HLA-DR molecules** on their surface and may also express **receptors for the C3b complement fragment.** However, they lack Fc receptors for IgG and have very few **C3d/EBV receptors.** Precursor B cells can also be recognized by their capacity to bind peanut agglutinin, a plant lectin that can also bind to immature T cells in the thymus and to activated B cells in the germinal centers of peripheral lymphoid tissues.

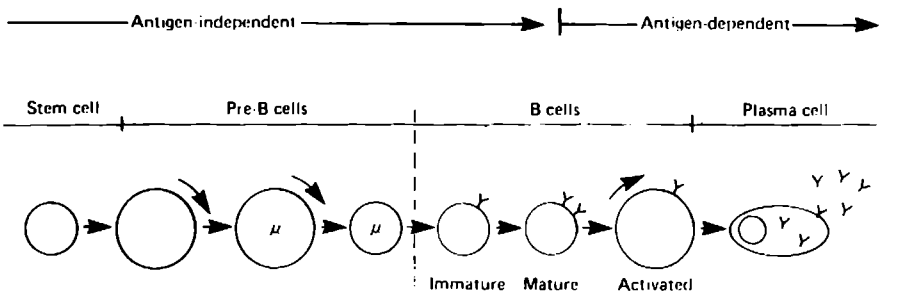

Figure 7–9. Stages in the life history of B cells. The antigen-independent development of stem cells into pre-B cells and the antigen-dependent development of B cells into plasma cells are shown. Those stages involved in proliferative activity are indicated by large circles and accompanying arrows. μ = IgM heavy chain; Y = complete immunoglobulin molecule with both heavy and light chains.

B Lymphocytes

B lymphocytes display immunoglobulins as integral proteins in their surface membranes. These membrane-bound immunoglobulins differ from secreted immunoglobulin molecules in several ways. For example, they possess a hydrophobic (transmembrane) sequence near the carboxyl terminus and have one less site for glycosylation than secretory immunoglobulin heavy chains.

Several stages in B lymphocyte differentiation can be defined by both phenotypic markers and functional capabilities. Immature B cells initially display membrane-bound IgM monomers on their surface, the density of which is significantly greater than on mature B cells, which bear approximately 200,000 molecules of IgM per cell (Fig 7–10). IgD molecules are not expressed by newly formed B cells. Immature B lymphocytes respond negatively to cross-linkage of their surface IgM molecules. Thus, early exposure to a multivalent antigen may eliminate the responsive B cell clone or block subsequent differentiation, leading to nonresponsiveness or tolerance to that antigen.

As B cell development proceeds, IgD molecules appear on their surface membranes along with IgM and become the predominant membrane-bound immunoglobulin isotype. While mature, resting B lymphocytes demonstrate higher levels of surface IgD than IgM, B lymphocytes that have been preactivated by antigen with the aid of T cells *lose* surface IgD as they gain receptors for soluble growth and differentiation factors produced by activated T cells or other cell types.

Immature B cells rapidly acquire receptors for **C3d/Epstein-Barr virus (EBV), C3b,** and the **Fc region of IgG molecules** (Fig 7–11). Histocompatibility antigens (HLA-A, -B, -C, and -D) are present on immature B lymphocytes. Activated B cells express increased amounts of D region (DR)-encoded molecules on their surface. Similarly, the density of both Fcγ and C3b receptors increases and then decreases during the terminal stages in B cell differentiation.

Activated B cells express several types of molecules that are not detected on resting B cells. Some of these activation molecules are also expressed on other types of activated or dividing cells. Examples are the interleukin-2 (IL-2) receptors, expressed also by activated T cells, and the transferrin receptors, which are expressed by all kinds of dividing cells. Other molecules detected as antigens on activated B cells are not seen on other types of cells. These are candidates for the receptors for growth and differentiation factors that have been identified in functional assays.

In addition to these receptors, B cells may exhibit fortuitous binding to other ligands, which can be used as cell markers. A subpopulation of human B cells form rosettes with mouse erythrocytes. This is a property of many B cells bearing IgM and IgD that is not exhibited by either pre-B or activated B cells. Other B cell surface components of interest include receptors for bacterial cell wall constituents and plant lectins. Binding of peanut agglutinin may be a useful marker for B cells in the germinal centers of lymphoid tissues as well as for bone marrow pre-B cells.

Monoclonal antibodies can be used to identify cell surface markers on human B lymphocytes. Such antibodies may react with all cells of B lineage or with surface molecules expressed only at certain stages in B cell differentiation. For example, monoclonal antibodies to the C3d receptor (which also serves as the EBV receptor) are reactive with mature B cells in both resting and activated states but appear unreactive with pre-B and mature plasma cells. Antigens detected on B cells may also be expressed by cells of other lineages, such as monocytes or T cell populations, but may still provide useful markers for functional B lymphocyte subpopulations. An example is the T10

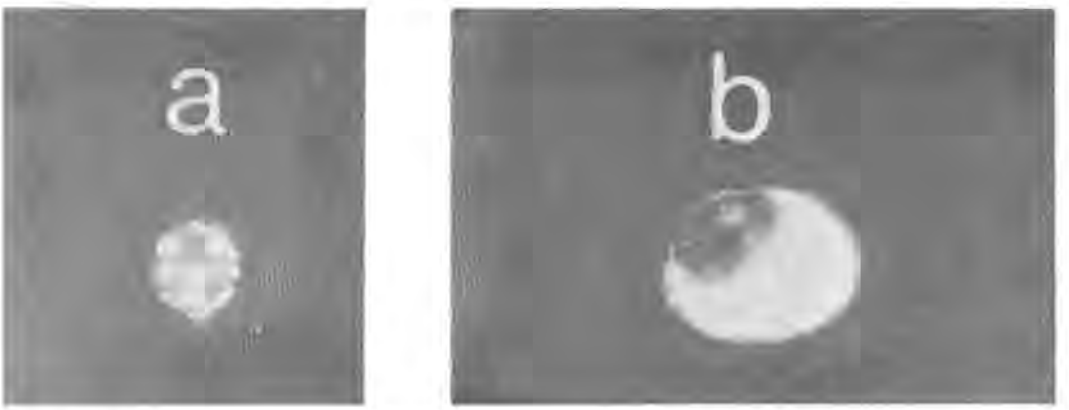

Figure 7–10. Immunofluorescent stain of a B lymphocyte *(a)* and a plasma cell *(b)*. A fluorescein-conjugated antihuman IgM was used to stain the membrane-bound IgM molecules on the cell in *(a)* and the cytoplasmic IgM within the cell in *(b)*. Note the patchy staining for the cross-linked IgM molecules on the B lymphocyte and the relatively homogeneous staining for cytoplasmic IgM in the plasma cell. The plasma cell is also larger than the B lymphocyte. (Magnification × 630.)

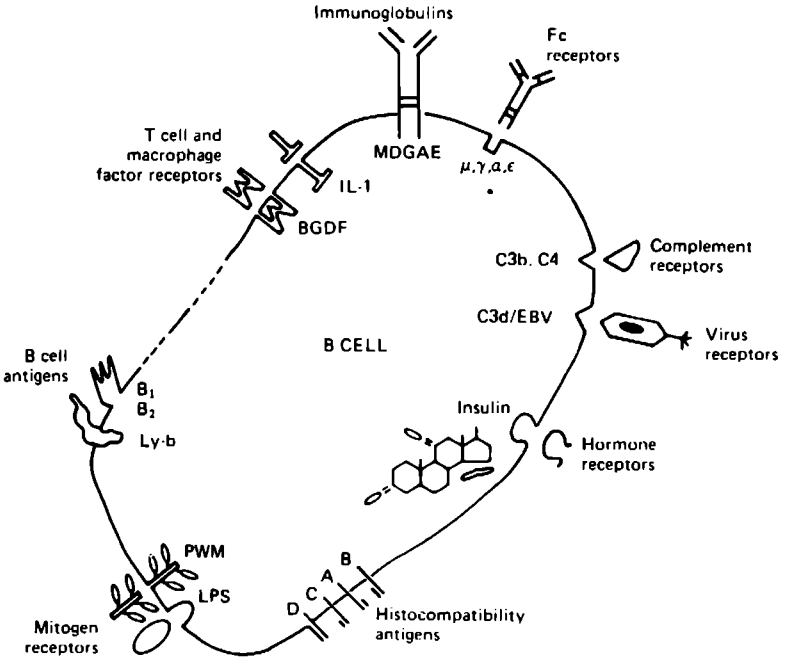

Figure 7–11. Receptors on the surface of a B cell. The letters M, D, G, A, and E stand for IgM, IgD, IgG, IgA, and IgE immunoglobulins, respectively. The notations μ, γ, α, and ϵ indicate receptors for the Fc portion of IgM, IgG, IgA, and IgE, respectively. C3b, C3d, and C4 indicate components of the complement pathway. EBV indicates receptors for Epstein-Barr virus. (The receptors for C3d and EBV reside on the same molecule.) B, A, C, and D stand for the histocompatibility antigens HLA-B, HLA-A, HLA-C, and HLA-D. PWM = pokeweed mitogen; LPS = lipoprotein polysaccharide; BGDF = B cell growth and differentiation factors.

glycoprotein of approximately 35,000 MW expressed by some B and non-B cells. Within the B lineage, expression of this antigen is restricted to pre-B, immature B, and plasma cells; mature B cells do not express detectable T10 antigen.

After antigen or mitogen stimulation, B lymphocytes can proceed along either of 2 branches of the pathway. They can differentiate (with or without cell division) into plasma cells that secrete large amounts of immunoglobulin, or they can divide and then return to a resting state as small, postmitotic B lymphocytes (Fig 7–12). The latter are called memory B cells, since they can rapidly differentiate into plasma cells following a second exposure to the same antigen. Activation of B cells provokes loss of membrane IgD, which apparently is not resynthesized by cells entering the memory cell pool; both Fcγ and C3b receptors are greatly diminished on external membranes, whereas those for C3d/EBV are not significantly affected.

Plasma Cells

Plasma cells represent the terminally differentiated state of high-rate antibody production and secretion toward which all B cells strive. The antibody-forming machinery of such cells is turned up full force. Over 40% of the total proteins plasma cells produce and secrete may be immunoglobulins, so that a single plasma cell can release thousands of antibody molecules per second.

A cell type known as a plasmablast can be described both morphologically and functionally as existing between the activated lymphocyte (or B lymphoblast) and plasma cell stages. The cells are large, with a greater nuclear:cytoplasmic ratio than plasma cells. Unlike B lymphoblasts, they actively secrete immunoglobulin, although at a lower rate than mature plasma cells. Such plasmablasts may contribute a large percentage of the antibody-secreting cells present during the early phases of antibody responses. Membrane-bound immunoglobulins and Fcγ receptors are present on plasmablasts, and cells of this phenotype form the majority of immunoglobulin-secreting cells found after EBV infection.

Plasma cells are recognized cytologically by their ovoid shape, eccentric spoke-wheel nucleus, and intensely basophilic cytoplasm. When stained with fluorochrome-labeled antibodies to immunoglobulin determinants, fixed plasma cells display intense fluorescence throughout their cytoplasm (Fig 7–10). Membrane-bound immunoglobulin and DR molecules are scant, and receptors for Fcγ, C3b, C3d, or Epstein-Barr virus are generally undetectable on mature plasma cells. The terminally differentiated plasma cell seldom divides and has an average life span of less

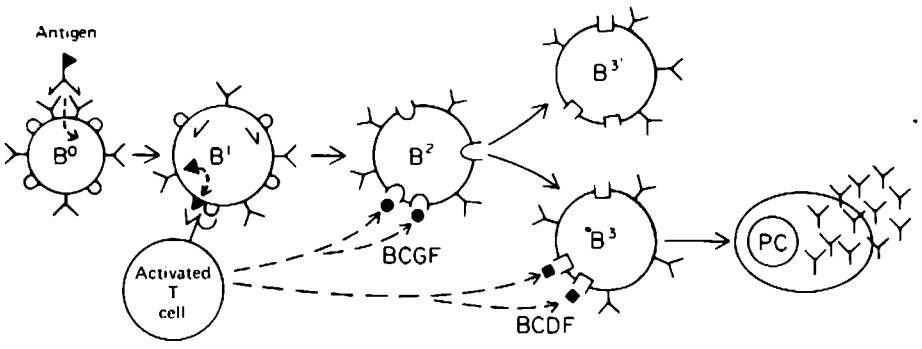

Figure 7–12. Minimal model for B cell activation and differentiation with T cell help. Black circles represent B cell growth factors (BCGF), and the BCGF receptors are depicted by open semicircles. Black squares represent B cell differentiation factors (BCDF), and the BCDF receptors are depicted by open squares. B^0, B^1, B^2, B^3, and $B^{3'}$ indicate distinctive stages of B cell differentiation. PC = plasma cell. ⋀ = multivalent antigen. ▲= processed fragment of antigen.

than 4 days. Few plasmablasts and mature plasma cells are found in the circulation; most are present in lymphoid tissues—the medullary cords of lymph nodes, red pulp areas of the spleen, lamina propria of the intestinal and respiratory tracts, and bone marrow sinusoids.

ACTIVATION OF B LYMPHOCYTES

The formation of pre-B cells and their B cell progeny does not require stimulation by antigens or other environmental stimuli. On the other hand, antigens and other mitogens are essential in inducing the resting B cells to begin proliferation and further differentiation.

B cell activation can be triggered by a variety of stimuli. This is not surprising, since B cells express a wide assortment of cell surface receptors, many of which can bind multiple ligands. Any multivalent ligand that cross-links immunoglobulin molecules can induce resting B cells to enter the cell cycle ($G_0 \rightarrow G_1$), enlarge, and begin DNA synthesis ($G_1 \rightarrow S$) in preparation for cell division ($S \rightarrow M$). Immunoglobulin cross-linking ligands include multivalent antigens, antibodies to immunoglobulin determinants, and protein A of staphylococci. Other ligands that can activate B cells are soluble T cell products, lipopolysaccharides (LPS) of gram-negative bacteria, EBV, *Chlamydia trachomatis*, *Nocardia opaca* mitogen, and phorbol esters. These ligands, alone or together, can induce B cells to proliferate, to undergo plasma cell differentiation, or to do both. For example, cross-linkage of surface immunoglobulin on mature, resting B cells with anti-immunoglobulin antibodies induces proliferation but not plasma cell differentiation. In contrast, EBV and LPS may induce both B cell proliferation and differentiation.

Interaction of a particular ligand and its B cell receptor can have different consequences depending on the stage in B cell differentiation when this interaction occurs. A good example of this is the cross-linkage of surface immunoglobulin by anti-immunoglobulin antibody. When this happens on a newly formed B cell, it aborts further development. The same cell surface signal activates the mature B cell as outlined above. On the other hand, immunoglobulin cross-linkage at a later stage completely blocks plasma cell differentiation even in the presence of all the appropriate inducing signals.

The interaction of a multivalent ligand with cell surface immunoglobulin triggers a series of metabolic events in B cells. These include activation of a membrane-associated enzyme, phospholipase C, which induces the turnover of membrane phosphatidylinositides (inositol phospholipids). The breakdown of phosphatidylinositol bisphosphate into inositol triphosphate and diacylglycerol leads to Ca^{2+} mobilization and to the activation of protein kinase C. The mobilized Ca^{2+} and diacylglycerol thus appear to function as second messengers in the transduction of extracellular signals into intracellular responses. Other inducing agents may bypass or short-circuit this B cell activation pathway. Although both LPS and phorbol esters are potent B cell activators, neither induces phosphatidylinositide turnover or Ca^{2+} mobilization. The phorbol esters cross the cell membrane by diffusion, bind to protein kinase C, and directly activate this enzyme. LPS may also trigger B cells via direct or indirect activation of protein kinase C. However, LPS is an unusual inducing agent. In the presence of macrophages, it drives B cells to differentiate into mature plasma cells.

Not much is known about how external signals are actually transmitted back to the nucleus, but heightened transcription of specific genes has been demonstrated in activated B cells. One of these is the cellular *myc* gene, the transcription of which is transiently upregulated following B cell activation with immunoglobulin cross-linkers or other B cell mitogens. The *myc* gene product is thought to be involved in regula-

tion of cellular proliferation. B cells are also known to make nuclear proteins that bind to enhancer DNA sequences and thus influence transcription of neighboring immunoglobulin genes.

Activation of a mature, resting B cell via ligand interaction with appropriate cell surface receptors induces a series of changes in the cell surface. These are important because they govern the evolution of a B cell response. One of the earliest membrane changes is electrical depolarization, followed within a few hours by a sharp increase in the cell surface density of MHC class II molecules, which are key elements in the interaction between B and T cells. Later events include B cell acquisition of surface receptors for transferrin, IL-2, and other growth- and differentiation-promoting factors produced by activated T cells. Activated B cells acquire other surface elements that have been identified as cell surface antigens but whose structure and physiologic roles have yet to be defined. Some of these may be receptors that guide the special routes of migration and homing of activated B cells.

Additional molecules that appear to be important in the complex process of B cell activation include interleukin-1 (IL-1), which is a macrophage product, and gamma interferon (IFN γ), which is produced by activated T cells. Recent evidence indicates that activated B cells can produce some of their own growth factors. Glucocorticoids may enhance plasma cell diffferentiation of activated B cells. Although insulin receptors are present on some B cells, effects of this hormone on B cell function have not been demonstrated, and no clear evidence yet exists for direct involvement of growth hormone, thyroid hormone, or sex steroids on B cell growth and differentiation.

Antigen-induced responses of B cells involve both direct and indirect T cell help and include a cascade of signals initiated by binding of soluble ligands to B cell receptors (Fig 7–12). After binding to surface antibodies, multivalent antigens are internalized, processed via partial degradation, and the antigenic fragments are then recycled to the B cell surface, this time in association with MHC class II molecules. A combination of self MHC class II and processed antigen is what the T cell sees with its antigen receptor. This interaction between T and B cells appears to be stabilized by bonds formed between the CD4 molecules on helper T cells and a nonpolymorphic area on the MHC class II molecules of B cells. The activated T_H cell then produces soluble factors that bind to specific receptors on the activated B cell to give signals for growth and differentiation. In addition to IL-2 and IFN γ, T cells make **B cell growth factors (BCGF)** of 2 sizes: BCGF I, MW ~18,000, and BCGF II, MW~50,000. Activated T helper cells also produce **B cell differentiation factors (BCDF)** of similar molecular weights. Issues presently under study are whether or not BCGF and BCDF are the same molecule and the nature of their receptors on B cells. The role of the monokine IL-1 in B cell activation is also still unclear.

In a given antigen-initiated response, not all of the activated B cells will receive sufficient BCDF influence to complete terminal plasma cell differentiation. These B cells enter the memory pool and are responsible for the more rapid and heightened antibody response following a secondary exposure to the antigen.

The physiologic significance of polyclonal B cell activation is unclear. A large number of microorganisms (both gram-positive and gram-negative bacteria, intracellular bacteria, EBV, cytomegalovirus) induce differentiation of multiple clones of B cells and secretion of antibodies not necessarily directed toward the stimulating microbe itself. The resulting mixture of antibodies may include those with specificity for other noxious stimuli or agents that would affect disease progression. EBV can trigger human B lymphocytes to begin the secretion of antibodies to phosphorylcholine, a molecule not present on viral membranes but found in the cell wall of most strains of *Streptococcus pneumoniae*. Phosphorylcholine may induce polyclonal antibody synthesis itself, some of which is directed toward microbes lacking the phosphorylcholine molecule on their surfaces. It is possible that polyclonal B cell activation is of importance both during the early phases of illnesses due to infection and in various autoimmune phenomena.

ISOTYPE SWITCHING

All immature B cells express membrane-bound IgM, and most go on to express IgD encoded by the next downstream heavy chain constant region gene. So far, only differentiation of B cells into IgM plasma cells has been discussed. However, some members of each B cell clone undergo a switch in the heavy chain isotype they express. **Isotype switching** can be either sequential (eg, IgM to IgG3 to IgG1 and so on in the order of the C_H genes on the chromosome [see Chapter 5]) or direct, from IgM to any of the other isotypes encoded by downstream C_H genes, the order of which is $C\mu$, $C\delta$, $C\gamma_3$, $C\gamma_1$, $C\alpha_1$, $C\gamma_2$, $C\gamma_4$, $C\epsilon$, $C\alpha_2$. The most commonly used pathways are direct isotype switches from IgM, as illustrated in Fig 7–13. Isotype switching involves deletion of the C_H genes in front of the expressed IgG, IgE, or IgA gene. Hence, "backward" switches do not occur in normal cells.

Members of the B cell clone that undergo isotype switching continue to express the same light chain and V-D-J_H gene complex, so that the antigen specificity is not altered except by the occurrence of somatic mutations. The major biologic advantage of isotype switching is the elaboration of antibodies of the same specificity with a range of heavy chains with different biologic characteristics.

The mechanism of regulation of heavy chain isotype switching is still unclear. The initial switches of surface immunoglobulin isotypes occur as an inherent feature of immature B cell clones and do not require T cell influence or deliberate exposure to antigen. On the other hand, the nature of the antigen and T cell influence can determine the relative isotype distribution

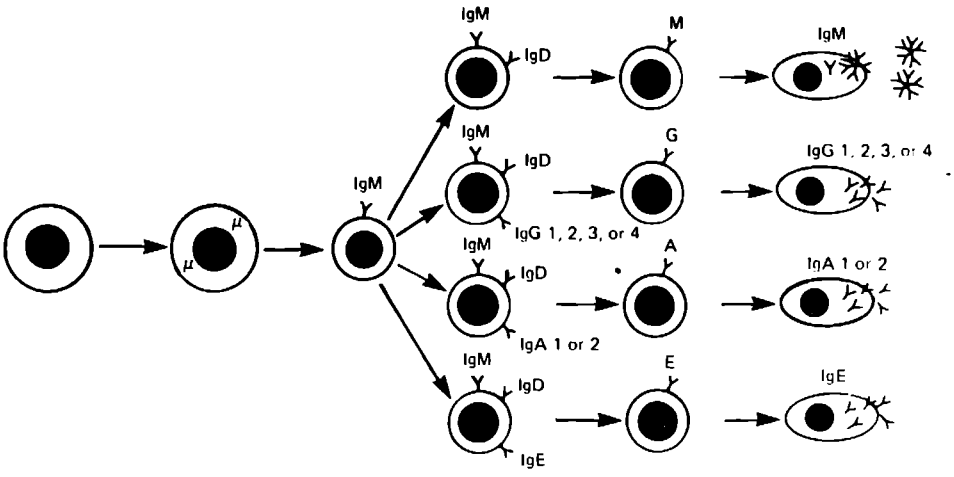

Figure 7–13. Heavy chain isotype switching by B cells. μ = the heavy chains of IgM. The arabic numbers following IgG and IgA indicate the subclasses of these 2 immunoglobulins.

in the plasma cell response. For example, polysaccharide antigens preferentially elicit IgG2-producing plasma cells, whereas with T cell help protein antigens usually elicit a predominance of IgG1 antibody producers. Whether antigens and T cells actually induce B cell isotype switching or instead preferentially induce proliferation and differentiation of switched B cells that are committed to synthesis of the different isotypes is still controversial. However, one way in which T cells can influence the isotype response pattern is via the production of isotype-specific binding factors.

ONTOGENY OF B CELL RESPONSIVENESS

Large numbers of B cells are produced daily from the eighth week of gestation onward, and the newborn appears to have a full complement of B cells of all isotypes (Fig 7–14). However, an adult pattern of antibody responsiveness is not acquired until several years after birth. While many factors may contribute to this developmental pattern, including the cumulative effects of exposure to antigens and other environmental stimuli, the reasons for the relative immunologic immaturity in infants are unclear.

Antibody responsiveness to different types of antigens is sequentially acquired during ontogeny. B cell responses to thymus-dependent antigens (eg, protein antigens) and certain thymus-independent antigens (eg, *Brucella abortus* bacterial antigens) can be induced in newborns, whereas antibody production in response to other thymus-independent antigens (eg, polysaccharides) is not observed until much later in development. For example, antibody responses to polysaccharide vaccines do not occur regularly until after 3 years of age, whereas excellent antibody responses to protein antigens can be induced within the first months of life.

The acquisition of surface immunoglobulin isotypes other than IgM and IgD begins early in fetal development (Figs 7–13 and 7–14). By the fourth month of gestation, some of the B cells exhibit either IgG or IgA together with IgM and IgD. At birth, most IgG- or IgA-bearing B cells still express IgM and IgD, whereas in adults most of the IgG- or IgA-bearing B cells display only the single surface isotype destined to be secreted by their mature plasma cell progeny.

B lymphocytes capable of developing into plasma cells that may secrete IgM, IgG, or IgA antibodies are present before birth, but an early preponderance of IgM producers is the rule. Certain types of infections occurring during intrauterine or early postnatal life (eg, rubella, syphilis, or chlamydial pneumonia) can induce production of IgG and IgA antibodies in addition to IgM antibodies. Thus, the ability to actively secrete immunoglobulin isotypes other than IgM is acquired by some members of the B-lineage during fetal life. However, when lymphocytes from newborn infants are placed in culture and appropriately stimulated, the immunoglobulin produced is almost entirely IgM regardless of the stimulus or the source of T cell help. EBV-transformed newborn cells produce IgM almost exclusively, whereas comparable EBV-infected B cells from adults give rise to cells synthesizing each of the major (μ, γ, α) heavy-chain classes. Most of the antibodies made following immunization of newborns with protein antigens are IgM; several weeks may be required before IgG antibodies predominate. The shift from IgM to IgG antibody production usually occurs within a few days after immunization of older children and adults.

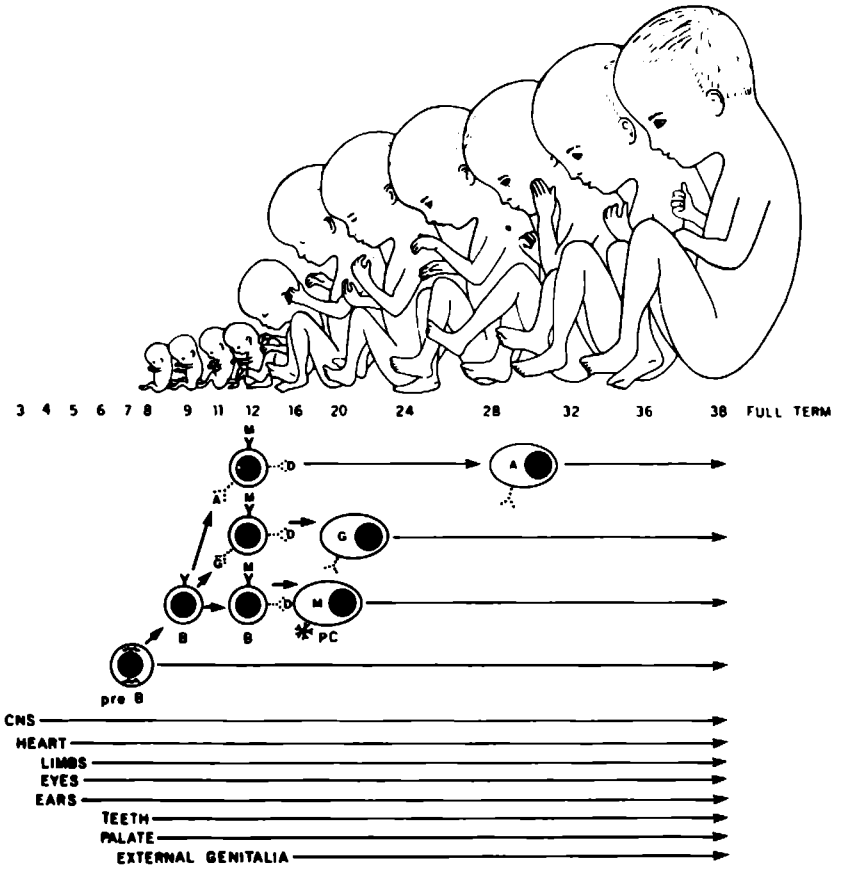

Figure 7–14. Comparative ontogeny of B cell development in human fetuses. The development of B cells and plasma cells is compared with the development of other organs. B = B cell; PC = plasma cell; M, D, G, and A = IgM, IgD, IgG, and IgA immunoglobulins, respectively.

B CELL DIFFERENTIATION & IMMUNODEFICIENCY DISEASES

The association with X-linked inheritance of several **B cell immunodeficiency syndromes** in both humans and mice suggests the presence of multiple genes on the X chromosome that encode for proteins essential to various steps in B cell differentiation (see Chapter 20). **Infantile X-linked agammaglobulinemia** is associated with a developmental arrest at the pre-B cell level. Normal numbers of pre-B cells are present, but relatively few B lymphocytes are formed in the bone marrow of affected boys. The limited numbers of B cells generated in individuals with X-linked agammaglobulinemia appear normal in their ability to undergo plasma cell differentiation. The defective gene apparently prevents the normal development of B cells from pre-B cells in affected males.

Another X-linked hypogammaglobulinemia syndrome is associated with short stature and a deficiency of pituitary growth hormone. The B cell abnormality is phenotypically similar to X-linked agammaglobulinemia with abortive development of B cells.

Hyper-IgM immunodeficiency, another X-linked syndrome, is characterized by a failure of B cells to undergo isotype switching. Normal numbers of IgM/IgD-bearing cells are present in the circulation of these patients, and these cells are fully capable of differentiation into IgM-secreting plasma cells. However, no other heavy-chain isotype is expressed by either B lymphocytes or plasma cells. This may suggest that a gene on the X chromosome encodes a protein involved in isotype switching.

Other primary defects in B cell differentiation have been more difficult to define and are often associated with abnormal T lymphocyte development or behavior. In an **X-linked form of severe combined immunodeficiency,** T cells are numerically deficient but B cells are not. Apparently as a secondary consequence, B cell differentiation is arrested at a mature B cell level.

An acquired form of panhypogammaglobulinemia,

called **common variable immunodeficiency,** is usually characterized by the production of normal numbers of clonally diverse B cells that fail to undergo plasma cell differentiation. The defect in most of these patients appears to be a flaw in the B cell activation pathway that is normally initiated by cross-linkage of surface immunoglobulin molecules.

Individuals with **selective IgA deficiency** usually possess IgA-bearing B lymphocytes, but they fail to undergo normal differentiation into IgA1- and IgA2-producing plasma cells. Although abnormalities in suppressor T cell function have been found in some IgA-deficient individuals, the basis for the regulatory defect in this relatively common immunodeficiency disease is still a mystery.

Lymphocyte development can be profoundly altered by absence of enzymes involved in purine nucleoside metabolism. Individuals with **adenosine deaminase deficiency** have few B or T cells, whereas B cells are affected to a much lesser degree than T lymphocytes by **nucleoside phosphorylase deficiency.** Stunted development of B and T cells may also reflect more generalized abnormalities in stem cell differentiation, as in reticular dysgenesis involving deficient development of B, T, and myeloid cell lines.

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8 Interleukins & Interferons

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Many hormonelike mediators are secreted in the course of immunologic and inflammatory reactions. Such mediators are generally termed "cytokines": Those produced by T and B lymphocytes are called "lymphokines," and those produced by monocytes or macrophages are called "monokines." These mediators function as intercellular signals that regulate local and, at times, systemic inflammatory responses. Cytokines modulate inflammation and immunity by regulating the growth, mobility, and differentiation of leukocytes as well as nonleukocytic cells.

This chapter will emphasize the more pivotal cytokines that amplify the afferent as well as efferent limbs of the immune response—namely, interleukin-1, interleukin-2, and interferons. These cytokines serve as endogenous second signals that act in sequence in conjunction with antigens to rapidly amplify both localized and systemic host defense mechanisms involving macrophages, lymphocytes, and other cell types.

INTERLEUKIN-1

In 1972, a **leukocyte-activating factor (LAF)** was discovered in the supernatant of cultures of adherent human peripheral blood cells and murine splenocytes that was mitogenic for murine thymocytes. Human LAF was also "comitogenic" in that it synergistically enhanced the proliferative response of murine thymocytes to polyclonal lectin stimulants such as concanavalin A (Con A) and phytohemagglutinin (PHA).

In 1974, it was reported that cultured human monocytes also secreted a **B cell-activating factor (BAF)** that stimulated antibody production by T cell-depleted murine splenocytes. Subsequent analyses showed that the biochemical properties of LAF and BAF were similar to each other and to a number of other macrophage activities that were identified by other acronyms. In 1979, these factors were renamed interleukin-1 (IL-1). IL-1-like factors are detected using bioassays of their comitogenic effect on thymocytes or certain cell lines. IL-1 can be distinguished from interleukin-2 (IL-2), the only other cytokine with thymocyte mitogenic activity, since IL-1 does not support the growth of IL-2-dependent lymphocyte cell lines.

Cell Sources of IL-1

The adherent cell source of IL-1 has been identified as monocytes or macrophages. All types of macrophages, including those from the peritoneal cavity, spleen, Kupffer cells of the liver, and alveolar macrophages from the lung, are capable of producing IL-1. However, since 1981 it has become apparent that IL-1-like factors are produced by virtually all nucleated cell types: keratinocytes, epithelial cells, astrocytes and microglial cells from the brain, mesangial cells from the kidney, human B cell lines and normal B lymphocytes, dendritic cells, Langerhans cells, melanoma cell lines, fibroblasts, endothelial cells, an OKM 1^+ subset of large granular lymphocytes (LGL), and neutrophils. IL-1-like factors may thus be produced by all cell types with the exception of red blood cells.

A number of cell lines produce low levels of IL-1 constitutively, and most normal cell types can be stimulated by a variety of agents to produce more IL-1. For example, keratinocyte lines produce IL-1 during the G_1 phase of the cell cycle. Stimulants that induce IL-1 production by other cell types include lipopolysaccharide (LPS) in the case of LGL, dendritic cells, astrocytes, microglial cells, and normal B cells and muramyl dipeptide (MDP) for fibroblasts, aluminum hydroxide for neutrophils, and LPS or *Staphylococcus albus* for Langerhans cells. Macrophages can be stimulated directly to increase their IL-1 production by particulate reagents such as silica or by adjuvants such as LPS or MDP. Alternatively, agents that activate lymphocytes indirectly stimulate macrophages to produce IL-1 by either an Ia-dependent, genetically restricted cell contact with activated lymphocytes or an unrestricted mediation by lymphokines such as colony-stimulating factors (CSF) or immune interferon (IFN γ) that stimulate macrophages. Although the mechanism of induction of IL-1 production is unknown, intracellular calcium presumably plays a role in IL-1 release, since the calcium ionophore A23187 is a potent enhancer of IL-1 production.

The nature of the stimulant influences whether IL-1 is predominantly accumulated at intracellular sites or is also released into the extracellular environment. Agents such as latex particles, LPS, and zymosan stimulate increases in both the intracellular and extracellular levels of IL-1, whereas silica particles and phorbol myristate acetate (PMA) predominantly stimulate the release of IL-1. The factors regulating the release of IL-1 are unclear, but cell injury has been suggested as one cause of increased release of IL-1. Conversely, antibodies to Ia (MHC class II) determi-

agents inhibit IL-1 release by silica- or LPS-stimulated murine macrophages but instead lead to an increase in the intracellular levels of IL-1 activity.

Inhibition of IL-1 Production

Only a few agents have been shown to inhibit IL-1 production by macrophages at pharmacologic concentrations. Hydrocortisone in the 10^{-5} to 10^{-7} mol/L range inhibits both the production and the comitogenic effect of IL-1. This inhibitory effect may account in part for the anti-inflammatory effects of steroids. Other immunosuppressive drugs such as cyclosporine that interfere with T lymphocyte function can suppress T cell-induced but not LPS-induced IL-1 production.

High doses of some macrophage stimulants unexpectedly yield suboptimal levels of IL-1 activity. In this case, IL-1 production can be restored by the addition of indomethacin, which is in itself not stimulatory but presumably acts to inhibit prostaglandin production. Indeed, direct addition of prostaglandin E_2 (PGE_2) interferes with IL-1 production in response to optimal doses of stimulants. Thus, products of the cyclooxygenase pathway such as PGE_2 exert a negative effect on IL-1 production. Conversely, since inhibitors of the lipoxygenase pathway reduce IL-1 release, leukotrienes may stimulate IL-1 production.

Several cell types also have been noted to inhibit production of IL-1 by cultured LGL. Removal of $T8^+$ lymphocytes by treatment with anti-T8 and complement can result in enhanced IL-1 production by LGL, suggesting that suppressor T cells may negatively regulate IL-1 production. Furthermore, spleen cells from guinea pigs desensitized with high doses of antigens inhibited the release of IL-1 by antigen-stimulated peritoneal cells. This suppression required both lymphocytes and macrophages and was not blocked by indomethacin.

Biochemical Properties of IL-1

IL-1 is a polypeptide based on its sensitivity to pronase, but its activity is not reduced by trypsin, pepsin, or chymotrypsin. There is no evidence that carbohydrate residues play a role in the bioactivity of IL-1. IL-1 is a relatively stable cytokine that survives temperatures from − 70 to + 56 °C and pH ranges from 3.0 to 11.0. Soluble IL-1 exhibits considerable molecular-weight heterogeneity. IL-1 is frequently detected as an aggregate in the 50,000- to 75,000-MW range. A 17,000-MW form represents the major stable released form of IL-1. Normal human urine also has been reported to contain heterogeneous forms of IL-1. A urinary 4000-MW IL-1 activity may be related to a 4000-MW fragment of IL-1 detected both in plasma and after trypsinization of the 17,000-MW IL-1.

The mRNA transcript of IL-1 cDNA codes for a 31,000-MW form of IL-1 that is also the predominant size of the inactive intracellular precursor of IL-1. Since the nucleotide sequence for IL-1 does not code for a typical signal peptide, IL-1 may be released from cells by a novel mechanism. It has therefore been proposed that posttranslational cleavage by the 31,000-MW intracellular precursor form of IL-1 yields the biologically active extracellular 17,000-MW IL-1.

IL-1 also exhibits charge heterogeneity and on isoelectrofocusing (IEF) yields 2 major peaks at about pI 7.0 and 5.0 in the case of human, pig, rat, mouse, and rabbit IL-1. The pI 5.0 IL-1 is the predominant form of murine IL-1, while the pI 7.0 form predominates in humans. Antibodies to these charged species of IL-1 show them to be antigenically distinct. Both human and murine macrophage-derived IL-1 have been purified and partially sequenced. Biologically active recombinant forms of IL-1 from both species have recently been produced. Two genes are thought to produce distinct IL-1 proteins that correspond to the pI 5.0 and 7.0 forms of IL-1. The predicted amino acid sequence of the murine pI 5.0 "IL-1α" exhibits only 20% homology with the human pI 7.0 "IL-1β" (Table 8–1). However, the nucleotide sequences of the pI 5.0 human IL-1 show 62% homology with murine pI 5.0 IL-1. In situ hybridization of metaphase chromosomes with IL-1β cDNA suggests that the gene for human IL-1β is located near the 2q14 region of the long arm of chromosome 2.

Regulation of IL-1 Gene Expression

Unstimulated monocytes spontaneously express low levels of both IL-1α and IL-1β mRNAs. The level of IL-1β mRNA begins to rise 1 hour after stimulation of human monocytes with LPS; reaches a maximum 6 hours after treatment; and achieves a level 40-fold over that in the unstimulated cells. In contrast, treatment with LPS increased the level of IL-1α mRNA only 2- to 3-fold over that of unstimulated monocytes.

Following LPS stimulation, the amount of IL-1α and IL-1β mRNA produced by human macrophages determines the relative amount of IL-1α and IL-1β protein produced by the cells. Estimates of the abundancies of mRNA for IL-1β and IL-1α in LPS-stimulated human monocytes are 0.1% and 0.01% of the total poly(A)$^+$ RNA, respectively.

Role of IL-1 in T Lymphocyte Activation

The capacity of IL-1 to augment thymocyte and to a lesser extent T lymphocyte growth presumably leads to expansion of the lymphocyte population. However, only a minor subset of thymocytes that does not express cell surface receptors for peanut agglutinin (PNA^-) is capable of responding to IL-1. These cells comprise the cortisone-resistant subset of immunocompetent thymocytes located in the thymic medulla and in the subcortical prethymocyte subset. The im-

Table 8–1. Classification of IL-1.

Biochemical Properties	Alpha	Beta
Intracellular MW	31,000	31,000
Extracellular MW	17,500	17,500
Extracellular IEF (pI)	5.0	7.0

IEF = isoelectric focusing.

mature subset of PNA^+ cortical thymocytes is unreactive to IL-1.

IL-1 promotes lymphocyte differentiation, as indicated by changes in phenotypic cell surface markers. Human IL-1 can augment the expression of "stable" sheep erythrocyte E rosette formation by human T lymphocytes. IL-1 decreases PNA binding by lectin-stimulated T lymphocytes. IL-1 increases both the viscosity of Lyt 1^+ murine lymphocyte membranes and their capacity to bind antigens. Increases in the accessibility of the antigen-binding receptors induced by the nonspecific IL-1 signal may increase antigen-specific immune responses.

IL-1 stimulates T lymphocyte functions and increases the production of lymphokines such as IL-2, CSF, B cell growth factor (BCGF), IFN γ, and lymphocyte-derived chemotactic factors (LDCF), each with their own biologic effects (Fig 8–1). In fact, even the thymocyte comitogenic effects of IL-1 are mediated by IL-2. This view is supported by experiments showing that monoclonal antibodies to IL-2 largely inhibit the proliferative responses of PNA^- thymocytes to IL-1 as well as to IL-2. Consequently, IL-1 acts as a differentiation factor for T cells.

MHC-histocompatible accessory cells such as macrophages are required to enable antigens to initiate T lymphocyte-mediated immunologic reactions. These accessory cells not only "present" antigen in a cell contact-dependent manner to the appropriate clone of T cells that bear complementary recognition (T cell receptor) sites but also produce IL-1, which promotes both proliferative and differentiative responses of activated T lymphocytes. It must be emphasized that IL-1 by itself cannot replace this requirement for antigen presentation by autologous or syngeneic (Ia- or DR-identical) macrophages. Lymphocytes depleted of macrophages cannot be activated by antigens even if supplemented by IL-1, and glutaraldehyde-fixed macrophages bearing antigens can activate T cells only if supplemented with IL-1. Both a cell contact-dependent macrophage-lymphocyte interaction and an amplifying IL-1 signal are required to obtain lymphocyte activation.

Based on the foregoing observations, a sequence of interactions of exogenous stimulants and interleukins during various stages of the cell cycle of lymphocytes can be proposed (Fig 8–1). Antigenic or polyclonal stimulants are processed and presented to T lymphocytes by accessory cells such as macrophages. This shifts lymphocytes from the resting G_0 state into the early G_1 phase of the cell cycle with concomitant synthesis of lipids, RNA, and proteins. IL-1 stimulates some of the lymphocytes to proceed further into the G_1 phase of the cell cycle and promotes their expression of IL-2 receptors. Other lymphocytes are stimulated by IL-1 to produce IL-2, which in turn stimulates IL-2 receptor-bearing lymphocytes to develop transferrin receptors and to proceed into the S phase of the cell cycle.

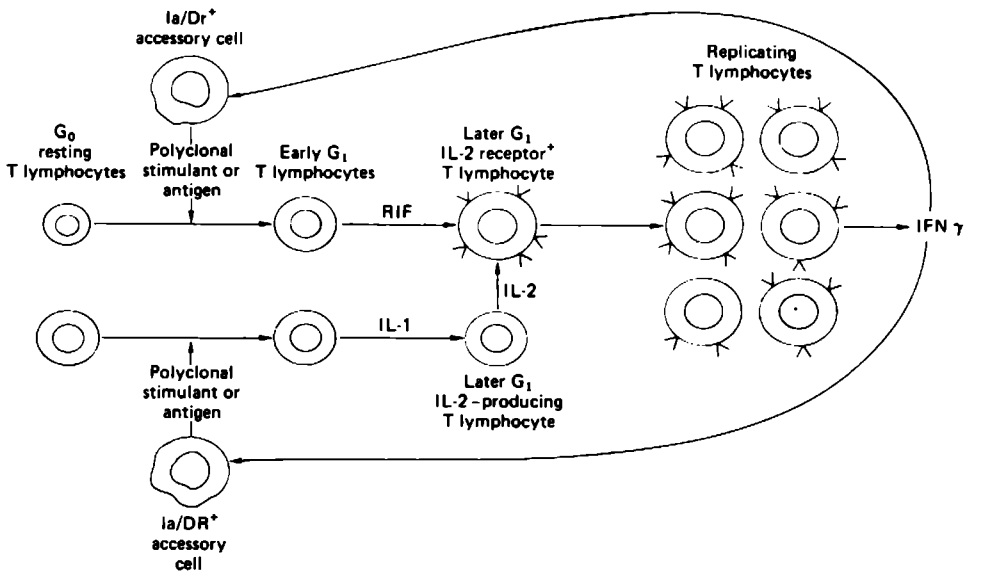

Figure 8–1. Role of IL-1 in the T lymphocyte cell cycle and lymphokine cascade. Following an initial activation signal by accessory cell presentation of antigen, resting (G_0) T cells proceed into the G_1 phase of the cell cycle. IL-1 augments expression of IL-2 receptors and production of a battery of lymphokines, including IL-2, CSF, LDCF, IFN γ, and BCGF. These intercellular signals further amplify the activities of appropriate target cells. IL-2 induces IL-2 receptor-bearing lymphocytes to enter S phase. IFN γ has a positive feedback effect on IL-1 production, antigen processing, and Ia/DR expression, thus promoting accessory cell functions. RIF = receptor-inducing factor.

Effects of IL-1 on Cytotoxic & Suppressive Lymphocytes

IL-1 promotes the specific cytotoxic functions of a subset of T lymphocytes (CTL). This effect of IL-1 on CTL may be mediated by increases in IL-2 production and IL-2 receptor expression as well as by direct differentiative effects of IL-1 on CTL. The capacity of lymphoid cells to manifest nonspecific killing of tumor cell targets has also been shown to be enhanced by IL-1. IL-1 augments the natural killer (NK) activity of human peripheral blood mononuclear cells in conjunction with IL-2 and IFN.

IL-1 may abrogate T lymphocyte suppressor activities. This is based on either increased helper or decreased suppressor cell activities. For example, addition of IL-1 has been shown (1) to restore lymphoproliferative responses that had been suppressed by anti Ia; (2) to result in lymphoproliferative responses by "nonresponder" mice to a synthetic antigen; and (3) to alleviate the immunosuppressive effects of ultraviolet irradiation, which reduces both Ia expression and IL-1 production by murine accessory cells.

IL-1 may under other circumstances promote suppressor T cell activities. IL-1 enhances the generation of histamine- and Con A-induced suppressor T lymphocytes. Addition of IL-1 24 hours prior to an antigen actually interferes with the immune response. This suppressive effect of IL-1, like antigenic competition, may be mediated by the enhancement of nonspecific T suppressor cell activities.

Effects of IL-1 on B Lymphocytes

IL-1 augments the in vitro proliferation, differentiation, and antibody-producing functions of B lymphocytes. It also induces, in pre-B cells, expression of surface immunoglobulin receptors. IL-1 promotes B cell antibody production directly and also indirectly by promoting the functions of T helper cells such as the production of BCGF. Since B lymphocytes can both produce and react to IL-1-like factors, IL-1 may function as an autoregulatory signal for B cells.

Effects of IL-1 on Nonlymphocytic Cells

In addition to the immunologic activities discussed above, macrophage-derived IL-1 or closely related molecules promote the growth or functional activities of almost every nonlymphocytic cell type on which IL-1 has been tested (Table 8–2). These pleiotropic effects of IL-1 can account for a wide variety of manifestations of acute and chronic inflammatory reactions such as fever, elevation of acute phase proteins, changes in circulating levels of plasma metals, bone and cartilage resorption, anorexia, cachexia, leukocytosis, somnolence, leukocytic infiltration of inflammatory sites, and possibly augmented host resistance to tumors.

Mechanism of Action of IL-1

^{125}I labeling of purified recombinant IL-1 has been used to identify surface binding sites. A wide variety of cell types have a low number of binding sites for IL-1, fibroblasts having 1500–5000 sites per cell. The LBRM 33/5A IL-1-dependent T cell line binds about 500 sites per cell with an affinity of approximately $0.2–2 \times 10^{-10}$ mol/L at 8 °C. Treatment of surface-bound ^{125}I-IL-1 with bivalent water-soluble cross-linkers identified a membrane polypeptide with a molecular weight of 79,500 to which IL-1 was cross-linked.

As for postreceptor events, IL-1 induces metabolism of arachidonic acid in a number of cell types. Cyclooxygenase inhibitors such as aspirin and in-

Table 8–2. Target cells and actions of IL-1 and related substances.

Name/ Acronym	Target Cell	Activities
LAF	Thymocytes	Enhanced proliferation (IL-2–mediated)
LAF	T lymphocytes	Enhanced proliferation (IL-2–mediated) Lymphokine production Cytocidal activation of CTL
BAF	B lymphocytes	Enhanced proliferation Augmented antibody secretion Membrane immunoglobulin receptor expression
EP	Hypothalamus Central nervous system	Prostaglandin-induced fever Somnolence and anorexia
LEM	Hepatocytes	Elevation of acute-phase proteins Decreased plasma iron and zinc Increased plasma copper
LEM	Neutrophils	Release from bone marrow Chemotactic mobilization Release of lysozyme, lactoferrin, and specific granules Metabolic activation
PIF	Muscle	Prostaglandin-mediated proteolysis
Catabolin	Chondrocytes	Cartilage matrix breakdown Prostaglandin, collagenase, proteoglycanase, and plasminogen activator production
Catabolin	Synovial cells	Proliferation and production of collagen, prostaglandins, and plasminogen activator
IL-1	Osteoclasts	Resorption of bone Production of prostaglandins and collagenase
IL-1	Osteoblasts	Proliferation and production of collagen and prostaglandins
IL-1	Epithelial cells	Proliferation and secretion of collagen type IV
IL-1	Endothelial cells	Proliferation and production of thromboxane and procoagulant activity
IL-1	Fibroblasts	Proliferation and production of prostaglandins and collagenase
IL-1	OKM T$^+$ LGL	Increased NK activity
IL-1	Monocytes	Chemotactic mobilization Prostaglandin-mediated cytocidal activation

domethacin block IL-1/EP-induced production of PGE by hypothalamic and brain cells, muscle cells, and macrophages. Conversely, inhibitors of the lipoxygenase pathway partially inhibit the amplifying effects of IL-1 on T lymphocyte activation, suggesting that leukotrienes may mediate some of the effects of IL-1.

Inhibitors of IL-1 Activities

A number of agents interfere with the effects of IL-1 on lymphocytes, such as IL-1-mediated enhancement of IL-2 production—including hydrocortisone, an inhibitory urine protein, peptide factors produced by cell lines, and some serum proteins. Perhaps cyclooxygenase blockers, which inhibit only pyrogenic PGE-mediated effects, are less immunosuppressive and anti-inflammatory in their effects than corticosteroids, because the latter actually interfere with both the production and actions of IL-1. The urinary inhibitor of IL-1 activity has a molecular weight between 20,000 and 30,000 and becomes elevated with fever. Heterogeneous inhibitory peptides have been reported to be produced by different tumor cell lines that block the effects of IL-1 on lymphocytes rather than on fibroblasts, but some of these factors block the activities of both IL-1 and IL-2. The tumor cell inhibitors exhibit molecular weight heterogeneity and heat lability.

Other agents, including cyclosporine and PGE, inhibit the actions of both IL-1 and IL-2. Heterologous antisera that neutralize the biologic activities of murine and human IL-1 have been obtained. The identification of antagonists to interleukins may provide therapeutic means of controlling the effects of IL-1.

INTERLEUKIN-2

In 1976, a polypeptide hormone was described that induced T lymphocytes to proliferate and enabled normal T lymphocytes to be maintained continuously in culture. The ability of this **T cell growth factor (TCGF)** to support the continuous growth of normal T cell lines provided a rapid, reproducible, and specific bioassay for this lymphokine.

Since previously described mitogenic lymphokines such as thymocyte mitogenic factor, killer helper factor, and lymphocyte mitogenic factor all cochromatographed with TCGF, these biologic activities were renamed interleukin-2 (IL-2). IL-2 has been purified, and the gene responsible for its expression has been cloned. Thus, IL-2 activity can be ascribed to a single protein.

T Cell Biology & Development of Immune Reactivity

Mature T lymphocytes are in a resting G_0 state. When activated by a polyclonal stimulant or antigen in the presence of an Ia/DR-compatible accessory cell, the T cell is stimulated to enter the G_1 phase of the cell cycle, which is characterized by an increase in cell size and a rapid elevation of the transcriptional activity of the cell, leading to the acquisition of IL-2 receptors. In late G_1, T cells produce and secrete IL-2. In some situations, IL-1 has been shown to augment IL-2 production and IL-2 receptor expression. Many interactions between IL-2 and its cell surface receptor are required for the cell to progress into S phase and divide (Fig 8–1). It is important to note that IL-2 regulation of growth is nonspecific in that any cell possessing IL-2 receptors will be stimulated to grow by IL-2. Thus, immune specificity resides in antigen recognition and not in the growth factor response. Activated T cells secrete numerous humoral factors that regulate the immune response.

Cellular Sources of IL-2

Studies of isolated subpopulations of lymphocytes have revealed that antigen-induced IL-2 is largely produced by the "helper T" subset (human T4 lymphocytes). However, in response to appropriate stimuli such as PHA and class I MHC alloantigens, the T suppressor/cytotoxic subset of lymphocytes (human T8 lymphocytes) also produces IL-2. The immunocompetent thymocytes present in the thymic medulla and a subset of LGL displaying OKT 11 and Leu-11 surface markers can also be induced by PHA to produce IL-2.

Intracellular Regulation of IL-2 Production

Freshly isolated resting T cells do not contain IL-2 mRNA and do not spontaneously produce IL-2; but when activated with antigenic stimuli, mRNA for IL-2 can be detected by 1 hour, reaches a peak by 6–8 hours, and declines to baseline levels by 24 hours. IL-2 mRNA processing by stimulated normal lymphocytes is normally regulated by a labile repressive peptide, because treatment of the lymphocytes with cycloheximide results in greatly enhanced production of mRNA for IL-2. Since cycloheximide by itself does not lead to IL-2 mRNA production, it may function by blocking production of an inhibitor of the processing of heteronuclear RNA, which is a precursor for cytoplasmic mRNA production. Subsequent to processing and translation of IL-2 mRNA, most of the activity for IL-2 in a wheat germ translation system translocates to heterologous functional microsomes and becomes trypsin-insensitive, suggesting that it is located in the microsomal spaces. This leads to secretion of IL-2. IL-2 first appears in measurable levels extracellularly within 4–6 hours after stimulation, and peak levels are attained in 12–24 hours.

Modulation of IL-2 Production

IL-2 production by Con A-activated T cells can be inhibited by human suppressor T cells, suggesting that some suppressor T cells down-regulate immune responses by shutting off IL-2 production. In addition, a number of immunosuppressive agents exert their effects primarily by inhibiting IL-2 production. These include glucocorticosteroids, cyclosporine, and

PGE_2. PGE_2 probably inhibits accessory cell function, while the others act directly on the IL-2-producing T lymphocyte. Studies with dexamethasone-sensitive clones of a primate T lymphocyte line that constitutively produces IL-2 have shown that IL-2 secretion is completely inhibited after 16 hours of treatment with dexamethasone. Dexamethasone and cyclosporine have been reported to inhibit gene expression for IL-2 at the levels of mRNA transcription.

Besides IL-1, a variety of physiologic and nonphysiologic agents also augment IL-2 production. They include PMA, vasopressin and other neurohormones, hydroxyurea, sodium azide, and numerous cell surface-active compounds. Production of IL-2 seems to occur mostly during the G_1 phase of the cell cycle (Fig 8–1). These compounds act by decreasing cellular proliferation and thus prolonging the G_1 phase.

Molecular Properties of IL-2

Human, primate, and murine IL-2 have been purified to homogeneity. IL-2 is a single protein with a molecular weight of 15,000 that is variably glycosylated (Table 8–3). The presence of significant amounts of carbohydrates results in higher molecular weights and lower pI forms of IL-2. Since recombinant IL-2, which lacks carbohydrate groups, is as active as "natural" IL-2, carbohydrates are not necessary for activity of IL-2, at least in vitro.

Molecular studies using cloned cDNA from various human sources and other species indicate that there is only a single gene for IL-2. Its genomic structure has the coding region present in 3 exons. Chromosome mapping using hybrids between a mouse and the human line, which is a constitutive producer of IL-2, showed that the IL-2 gene is on chromosome 4. There is little or no homology between the sequence of IL-2 and that of other sequenced growth factors.

Receptors for IL-2

Resting lymphocytes do not respond to IL-2. The development of the IL-2-responsive state requires de novo acquisition of membrane receptors for IL-2. This concept is supported by the use of a monoclonal antibody (anti-Tac) that reacts with the human IL-2 receptor, suppressing IL-2-mediated growth of previously activated T cell lines and blocking activation of peripheral blood lymphocytes by antigens or lectins. This is associated with the ability of anti-Tac to block binding of radiolabeled IL-2 to cloned T cell lines. Anti-Tac has permitted the purification and characterization of the receptors for IL-2. The receptor is a glycoprotein of MW 55,000. The IL-2 receptor has been cloned and sequenced, and cDNAs for this receptor have been expressed. One gene, present on chromosome 10, codes for the IL-2 receptor, but multiple species (2 in humans, 4 in rodents) of mRNAs differing in their polyadenylation signals have been identified.

IL-2 receptors satisfy all the criteria of hormone receptors, including (1) a high-affinity binding constant of about 10^{-12} mol/L; (2) saturable binding occurring within 20 minutes at 37 °C; (3) inhibition of binding by unlabeled IL-2 but not other growth factors or hormones; and (4) target cell specificity. A close correlation exists between the concentration of IL-2 giving rise to a proliferative response (16 pmol of free IL-2 induces half-maximal growth responses) and concentrations leading to significant binding (21 pmol of free IL-2 gives half-maximal binding for the same cell line). These results support the conclusion that the biologic effects of IL-2 are initiated through binding to a specific receptor (Table 8–4).

Recently, using 1000-fold more IL-2 in binding assays, it has been possible to identify a class of receptors with low affinity for IL-2 (2.8×10^{-8} mol/L). Transfection of the IL-2 receptor gene into non-T cells leads to expression of IL-2 receptors with only low affinity for IL-2. Although such low-affinity receptors bind IL-2 less well and do not mediate T cell growth responses, they react as well with anti-Tac antibody as the high-affinity receptors.

Upon appropriate activation, T lymphocytes develop IL-2 receptors by 6 hours. By 24 hours, over 50% of the cells express a full complement of receptors. However, in the absence of IL-2, no proliferation occurs. Antigen-activated lymphocytes develop maximal levels of receptors in 2–3 days, and IL-2 receptor expression declines to low or nondetectable levels by day 14. Consequently, even in the presence of IL-2, growth ceases and the cells arrest in G_1. Re-addition of

Table 8–3. Properties of human interleukin-2.

Biochemical properties
Sensitive to treatment with heat, trypsin, and neuraminidase
Stabilized by polyethylene glycol and albumin
MW 15,400
Isoelectric point glycosylated, 6.8–8.0; nonglycosylated, 8.0
Biologic properties
Origin: peripheral T cells, medullary thymocytes; subset of LGL
Target cell: Activated T and B cells, LGL
Biologic effects: binds to specific receptor, promotes entry into S phase, stimulates proliferation
Not species-specific

Table 8–4. Properties of the human IL-2 receptor.

Biochemical Properties	Biologic Properties
One gene	**High-affinity receptor**
Multiple mRNA species	Transiently expressed (inducible)
On chromosome 10	Modulated by antigen
Short intracytoplasmic tail	Stimulates protein kinase C activation
Located on cell membrane	Stimulates Ca^{2+} flux
Phosphorylated by protein kinase C	Mediates protein phosphorylation
Two affinities for IL-2	Activates T cell–specific genes
MW 50,000–55,000	Induces lymphokine production
	Stimulates S phase progression
	Low-affinity receptor
	Increase modulated by IL-2
	No defined function

the activating signal stimulates the appearance of optimal numbers of receptors on the cell surface, and the cells will again grow in the presence of IL-2. The receptor number per cell again declines in 4–7 days, awaiting further activation. Consequently, the cyclic nature of normal T cell growth in vivo is based on the requirements for stimuli to induce receptors for IL-2, which permits IL-2-mediated growth of T cell clones in vitro. Other signals that enhance the expression of high-affinity IL-2 receptors on the cell surface include IL-1 and a novel mediator from virally transformed human T cell lines called ADF. IL-2 itself has also been reported to stimulate transcription of IL-2 receptor gene, which leads to a preferential expression of low-affinity receptors for IL-2.

The IL-2-dependent normal murine T cell lines used to assay for IL-2 are actually variants that constantly express high levels of IL-2 receptors and therefore need only IL-2 to grow indefinitely. In the absence of IL-2 the cells cease to proliferate, dying within 12–24 hours. These cell lines provide an assay for IL-2 that requires only 1 day and yields a marked difference in proliferation in the presence or absence of IL-2. The magnitude of proliferation is dependent upon the concentration of IL-2 present in the assay sample. A unit of IL-2 activity per milliliter is defined as the level of IL-2 activity that yields 50% of maximal stimulation obtainable with IL-2. This assay is rapid, quantitative, reproducible, and specific for IL-2.

T Cell Targets & Activities of IL-2

All subsets of peripheral T lymphocytes as well as medullary thymocytes can develop IL-2 receptors with high affinity and respond to IL-2. T cell subsets can also produce IL-2. Thus, T cells grow by an autocrine mechanism that is strictly regulated by external stimuli. However, it is not known whether the same T lymphocyte can produce and react to IL-2 at the same time or whether this occurs at different phases of the cell cycle. IL-2 receptor-bearing thymocytes will proliferate in response to IL-2 in conjunction with a comitogenic lectin. Also, the young adult athymic mouse has precursors for the IL-2-responder cell but not for the IL-2-producer cell. As a result, immunoincompetent thymocytes are capable of proliferation, but since they lack the ability to produce and release IL-2, they do not proliferate.

Function & Specificity of IL-2-Dependent T Cell Clones

The addition of IL-2 to purified activated T cells promotes several cellular functions prior to the onset of proliferation. IL-2-dependent T cells—predominantly helper T cells—produce other lymphokines, including IFN, CSF, interleukin-3, lymphotoxin, BCGF, and LDCF. IL-2-activated T cells can also exhibit enhanced cytotoxicity despite inhibition of DNA synthesis by mitomycin C. These results suggest that IL-2 promotes lymphocyte functions in addition to stimulating growth. These functions are amplified by the ability of IL-2 to induce clonal expansion of functionally activated cells.

T lymphocytes obtained after primary or secondary antigenic stimulation in vitro can be cloned in the presence of the original antigen, irradiated feeder cells, and IL-2. The frequency of clones obtained from unsensitized cells is much lower than that from cells previously stimulated with antigen. The cell clones maintained with feeder cells, antigen, and IL-2 remain normal in that they possess normal karyotypes, and the majority have been functionally stable for 1–2 years. This is probably the result of the constant reeducation of the cell by the antigenic stimulus. IL-2 preferentially supports the growth of CTL, but "helper" T cell lines also can be grown.

Populations of CTL generated by antigenic stimulation are specific in that they preferentially lyse target cells bearing the original stimulting antigens. However, low levels of nonspecific lytic activity for target cells that do not bear stimulating antigens can be detected. The ability of CTL populations to express a range of reactivities suggests that individual CTL receptors may recognize determinants shared (cross-reactive) with those of the stimulating antigens. It is noteworthy that cloned T cells have provided a homogeneous cell source for studies of the reactivity and biochemical nature of the T cell antigen receptors.

Clones of helper T cells have mediated delayed hypersensitivity responses when injected into histocompatible hosts along with specific antigen. Of interest was the fact that equivalent delayed hypersensitivity responses were observed when cloned T helper cells were inoculated into athymic mice and normal mice, suggesting that recruitment of host T cells was not necessary for the delayed hypersensitivity response and that the cloned helper T cells recruited host non-T cells as the delayed hypersensitivity-effector cells. Histologically, the nature of the cellular infiltrate in the delayed hypersensitivity responses mediated by cloned helper T cells in athymic and normal mice is indistinguishable from the Jones-Mote type delayed hypersensitivity response. Since the cells were presumably attracted by lymphocyte-derived chemotactic factors, cloned helper T cells are responsible for soliciting host inflammatory cell infiltration of the delayed hypersensitivity site.

Interactions of IL-2 With Non-T Cells

Although lacking T cell markers, LGL that exhibit nonspecific NK activity also respond to IL-2. Despite the fact that fresh LGL fail to react with anti-Tac antibodies, LGL are stimulated by IL-2 to grow, to produce other lymphokines, and to exhibit greater NK activity. The incidence of progenitors of 1:50 LGL that proliferate in response to IL-2 is lower than the 1:5 frequency of T cell progenitors. These data suggest that unstimulated LGL express either unique receptors for IL-2 that are not recognized by anti-Tac antibodies or that they express too few functional receptors to be detected. However, following in vitro incubation, LGL develop Tac-associated receptors for IL-2.

Binding of radiolabeled IL-2 to B lymphoblasts has also been reported by several groups. There are receptors with both high and low affinity for IL-2 on these B cells. Activated normal as well as some transformed human B lymphoblasts—but not resting B cells—express about 30% of the numbers of anti-Tac-reactive receptors for IL-2, as do activated T cells. IL-2 can induce increased antibody production as well as proliferation by purified B lymphocytes with somewhat higher (> 2- to 3-fold) doses of IL-2 than required for T cell responses. Finally, activated macrophages also express a low density of Tac-positive receptors for IL-2 and can be induced to become cytocidal by high doses of IL-2.

IL-2-IL-2 Receptor Binding; Intracellular Signaling

The mechanism by which the IL-2 signal is transmitted to the nucleus is being elucidated. Since activating signals such as phorbol esters, PHA, and antibody to the antigen receptor as well as IL-2 can induce transcription of the IL-2 receptor gene, it seemed likely that they shared some elements of the intracellular signaling process. The amino acid sequence of the IL-2 receptor reveals that unlike other growth factor receptors, it does not have an intracytoplasmic tyrosine kinase domain. In fact, it has an intracytoplasmic tail of only 13 amino acids. Many types of exogenous ligands activate protein kinase C through the ligand-receptor initiation of phosphoinositol diphosphate hydrolysis into diacylglycerol and inositol triphosphate, a mobilizer of intracellular Ca^{2+} (Fig 8–2). Phorbol esters bind to and activate protein kinase C directly, bypassing any requirement for phosphoinositol diphosphate hydrolysis. IL-2, PHA, PMA, or antireceptor each will induce the membrane association and activation of protein kinase C, suggesting that IL-2 and phorbol esters share components of a common intracellular mechanism of signal transmission. In addition, ligand binding to these receptors results in intracellular Ca^{2+} mobilization. These events occur less than 10 minutes after ligand binding. Both protein kinase C and Ca^{2+}-dependent calmodulin-associated kinase cause the phosphorylation of specific protein substrates, which may play a role in gene activation and DNA replication (Fig 8–2). However, there must be differences in what signals are transmitted through different receptors, since triggering through the antigen receptor does not lead to cell division, while IL-2 receptor triggering does. Labeled IL-2 when absorbed by activated but not resting lymphocytes at 37 °C rapidly becomes resistant to pH 4.0 treatment, presumably as a result of internalization of the receptor-ligand complex. The IL-2 then undergoes lysosomal degradation ($t_{1/2}$ 70–80 minutes). Protein kinase C translocation from the lymphocyte cytosol to the membrane followed by phosphorylation of the IL-2 receptor is thought to be needed for such internalization to occur.

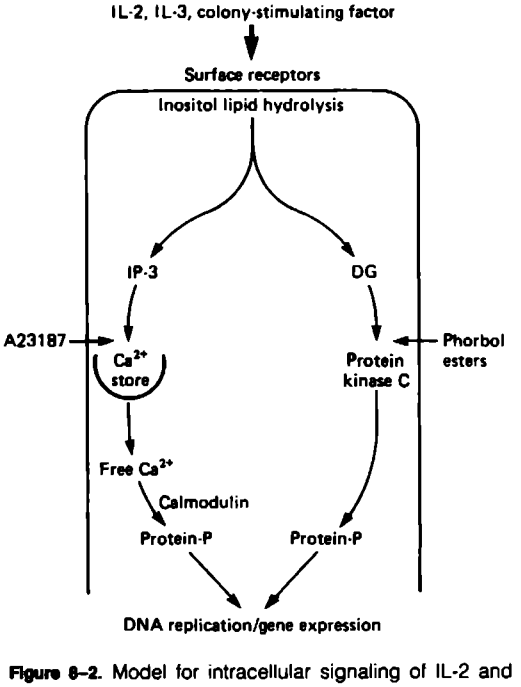

Figure 8–2. Model for intracellular signaling of IL-2 and other growth factors.

Abnormalities in Production & Responses to IL-2 & Its Receptor

Hyperproduction of IL-2 has rarely been detected. IL-2 is not normally present in serum or urine. IL-2 activity can be detected along with activated lymphocytes in the synovial fluid from inflamed joints of patients with rheumatoid arthritis. However, high levels of circulating IL-2 receptors are found in the sera of patients with mature T cell neoplasias, particularly those associated with the human T cell leukemia retrovirus.

Hypoproduction of IL-2 is a predictable finding in diseases associated with cell-mediated immunodeficiencies involving depressed T lymphocyte functions. Impaired IL-2 production has been reported in a variety of syndromes such as systemic lupus erythematosus, advanced metastatic cancers, acquired immunodeficiency syndrome (AIDS), and primary immunodeficiency diseases. The athymic nude mouse, which is deficient in immunocompetent T lymphocytes, has provided an excellent model for studying immunodeficiency due to defective IL-2 production. Interestingly, incubation of nude lymphocytes with IL-2 results in a lymphoproliferative response and the generation of CTL. Thus, nude mouse lymphocytes bear receptors for IL-2 and can respond to IL-2. Impaired in vitro PHA responses of lymphocytes from patients with cell-mediated immunodeficiencies and a gross imbalance in the ratio of T4 helper to T8 suppressor/cytotoxic lymphocytes can similarly be restored to normal by the addition of IL-2.

Although the lymphocytes from such patients are defective in producing IL-2, they are presumably able to acquire receptors for IL-2 and can respond to IL-2. In contrast, addition of IL-2 does not augment the normal lymphoproliferative response to PHA of lymphocytes from patients with X-linked or common variable forms of hypogammaglobulinemia. IL-2 has had only limited beneficial effects on the response of lymphocytes from patients with AIDS. Unfortunately, in vivo administration of IL-2 to patients with AIDS has had no beneficial effects.

In Vivo Effects of IL-2

IL-2 administration dramatically diminishes the survival of vascularized cardiac allografts in T cell-deprived rats. The IL-2 acts predominantly on the CTL component of the response.

Studies on the fate of IL-2 in vivo reveal that intravenously injected purified IL-2 is rapidly cleared from the circulation of humans or mice, with a half-life of 3–22 minutes. It is not clear whether part of the clearance is due to absorption by activated T cells. The kidney is the main site of IL-2 clearance. However, ligation of renal arteries prolongs the serum half-life only transiently. Only breakdown products of IL-2 can be recovered in the urine. Continuous intravenous infusion results in sustained levels of IL-2. Higher intravenous doses of recombinant IL-2 have multiple toxic side effects and result in elevation of serum ACTH and cortisol levels, which depresses the immune system. Intraperitoneal and subcutaneous injections of IL-2 lead to prolonged IL-2 serum titers of more than 2 units/mL at about 2 and 6 hours, respectively.

Significant enhancement of in vivo host antitumor activities by IL-2 has been reported. In the mouse and in humans, purified IL-2 augments in vivo NK responses and alloantigen responses and corrects immunodeficiency states, supporting the concept that purified IL-2 can function as an immune response modifier. In vivo administration of IL-2 by itself has had some effects in inhibiting tumor cell growth and metastasis.

The effect of adoptively transferred lymphocytes that have been sensitized in vitro with IL-2 has also been evaluated. Over 95% of the intravenously administered cells are cleared from the circulation within 20 minutes, with most of the cells cleared from the lungs, liver, and spleen. However, sufficient numbers of cells remain to mediate some immune functions. For example, cloned helper T cells are capable of restoring the antibody-secreting response to T cell-dependent antigens in nude mice.

In humans, the use of nonspecifically sensitized cells in adoptive immunotherapy has been investigated. Fresh lymphoid cells incubated in IL-2 develop the ability to lyse different fresh tumor cells but not fresh normal lymphoid cells. Further studies have shown that virtually any modified cell (eg, lectin-activated lymphoblasts) can by lysed. These **lymphokine-activated killer cells (LAK)** differ from the other cytotoxic lymphoid effector cells (NK and CTL) by this broader range of target cells. In murine models, these LAK cells have been shown to stimulate regression of metastases using several tumor systems. Recently, a phase I study utilizing the infusion of massive amounts (up to 10^{11} cells) of LAK cells activated with human recombinant IL-2 has shown that such a procedure is well tolerated in patients. Fever and chills were seen in all patients and headaches, nausea, and vomiting in some. Indium 111 cell-tracking studies show that the cells eventually are located in the spleen, lungs, and liver; but by the sixth infusion these cells are detected in the peripheral blood, with accumulation at tumor sites. Some tumor regression is observed only when LAK cells are given in conjunction with the maximally tolerated dose of IL-2, probably to augment cytolytic effector function. Unfortunately, administration of IL-2 results in greater toxicity than LAK cells alone. Combination therapeutic approaches involving LAK offer promise in attempting to eradicate established tumors in humans.

INTERFERONS

The interferons consist of a family of secreted proteins characterized by their ability to induce an antiviral state in almost all cell types. The interferons are produced by the cells of most vertebrates in response to specific stimuli. In 1957, Isaacs and Lindenmann discovered that a soluble factor produced by tissues exposed to inactive virus was able to transfer "interference" of viral replication to fresh tissues. They named the soluble factor interferon. Subsequently, it has been demonstrated that this antiviral activity resides in several proteins. Moreover, the same proteins that induce an antiviral state also have potent antiproliferative and immunomodulatory activities. The recent cloning of several of the interferon genes and the purification of the interferons to homogeneity have made it possible to attribute various biologic activities to the interferons with certainty and to begin elucidation of molecular mechanisms in the actions of the interferons.

Assays for Interferon

The commonly used assays for interferon (IFN) are antiviral bioassays that measure interferon-induced inhibition either of virus production by plaque assay or of the cytopathic effect of virus on cultured cell lines. In these assays, cells in culture are treated for 12–24 hours with varying dilutions of an interferon preparation followed by infection with an appropriate virus (usually encephalomyocarditis virus or vesicular stomatitis virus) at a suitable dilution. The antiviral effect is then measured after 24–48 hours. The titer in units is the reciprocal value of the highest dilution of the interferon solution giving a 50% decrease in the effect. Although these assays have recently been standardized by the use of international reference interferon standards, they nevertheless are time-consuming and imprecise. The development of radioim-

Table 8–5. Classification of interferons.

Interferon	Principal Cellular Source	Inducing Stimulus	Molecular Weight of Natural Monomeric Form	Glycosylated?
Type I				
α	Leukocytes	Virus or dsRNA	18,000–20,000	No
β	Fibroblasts	Virus or dsRNA	23,000	Yes
Type II (immune)				
γ	T lymphocytes	Mitogen, antigen, or lectin	20,000–25,000	Yes

munoassays has improved the quantitation of interferon concentrations and has facilitated identification of the type of interferon present.

Interferon Types & Induction

Many proteins with varying degrees of homology have the property of inducing an antiviral state in target cells and therefore are by definition interferons. The interferons can be divided into antigenically distinct types and classified according to their primary cell of origin or according to the stimulus for induction, as shown in Table 8–5.

Type I interferons (IFN α and IFN β) are induced by virus infections or artificially by a double-stranded RNA such as poly I:C. Most type I interferons are characterized by being stable at pH 2.0. IFN α, produced primarily by leukocytes, consists of multiple subspecies that are antigenically related. The classic human IFN α gene family has at least 15 functional nonallelic members. At least 8 different polypeptides with IFN α activity have been purified from crude interferon preparations induced by virus in human leukocytes. The amino acid sequences of these human IFN α subspecies are about 80% homologous. Recently, a second family of IFN α genes has been identified in the human genome. Besides the classic acid-stable subspecies of IFN α, other subspecies of IFN α, which are neutralized by anti-IFN α antibodies but are acid-labile, have been found in the serum of patients with autoimmune and immunodeficiency diseases.

The antigenically distinct IFN β is the major interferon synthesized by cells of solid tissues, including fibroblasts, but it can also be produced by leukocytes. There is one major human IFN β species, although minor species have also been reported. The amino acid sequence of IFN β is approximately 30% homologous with the IFN α family.

Type II interferon, also known as IFN γ or immune interferon, is produced during immune reactions by antigen-, mitogen-, or lectin-stimulated T lymphocytes. Type II interferon is labile at pH 2.0. This property is often used to identify a particular interferon as IFN γ. However, since some subspecies of IFN α are also acid-labile, data on acid lability must be combined with either antibody neutralization or biochemical data to identify the type of interferon present.

Although the reasons for the genetic diversity of the interferons in humans are not known, it should be pointed out that the different interferons are not equally potent in each of the activities attributed to interferon and the various types of interferon may be differentially active on different cell types.

Activities of the Interferons

The interferons induce an antiviral state that protects the target cells against most types of viruses. In addition, the interferons have potent cellular effects, some of which are listed in Table 8–6. The primary cellular effect is inhibition of cell proliferation. Interferons have been reported to both inhibit and enhance cell differentiation, depending on the cell type and dose of interferon. In some cases, these effects on differentiation are difficult to separate from the antiproliferative action of interferon. The interferons are also potent immunomodulatory agents. As is apparent, the interferon system is very complex, since not only are there many proteins with interferon activity but there are also many biologic effects of the interferons. As a result of this complexity, many conflicting responses to the interferons have been described, especially in the immune system. The observed effects of interferon depend on the type of interferon, the dose of interferon, the time of administration relative to the biologic effects being measured, and the presence of other immunomodulatory and hormonal factors.

Immunomodulatory Effects of Interferon

The immunomodulatory activities of interferon are

Table 8–6. Some effects of interferons on cellular functions.

Inhibits
Cell proliferation
Tumor growth
Fibroblast-adipocyte differentiation
Enhances
Promyelocytic and monoblastic leukemic cell differentiation
Phagocytosis by macrophages
Accessory cell functions of macrophages (IFN $\gamma > \alpha, \beta$)
Endotoxin-induced IL-1 secretion by macrophages (IFN $\gamma > \alpha, \beta$)
Generation of cytotoxic T lymphocytes
Activity of natural killer cells
Expression of cell surface histocompatibility antigens and Fc receptors
Mixed Effects
Erythroleukemic cell differentiation
Production of antibodies
Cell-mediated immunologic phenomena

mediated by the effects of interferon on the cells that carry out the host defense process, ie, macrophages, T and B lymphocytes, and LGL with NK activity.

A. Activation of Macrophages: The interferons increase bactericidal and tumoricidal capabilities of macrophages and augment their accessory cell functions. The interferons possess the activities described as **macrophage-activating factor (MAF)** and **monocyte migration inhibitory factor (MIF),** although other proteins may also have these activities. IFN γ is more potent in its MIF activity than IFN α or β.

Interferon-activated macrophages show morphologic evidence of maturation such as enlargement, increased spreading, pseudopod formation, and vacuolization beginning at 1 hour and peaking in 48–72 hours. The activation of macrophages by IFN α, β, or γ is accompanied by increased expression of receptors for the Fc portion of immunoglobulins (FcR). This increase in FcR expression promotes both increased phagocytosis of immune complexes and increased capacity of the macrophages to lyse antibody-coated bacteria, parasites, and tumor cells by antibody-dependent cell-mediated cytotoxicity (ADCC), in which antibody molecules couple macrophages to target cells by binding to their FcR and antigenic sites, respectively.

The induction of antigen-specific, T lymphocyte-mediated immune responses requires "presentation" of antigen in conjunction with a class II histocompatibility antigen (murine Ia antigen or the human equivalent DR antigen) by an accessory cell to a helper T lymphocyte. IFN γ and, to a much lesser degree, IFN α and β induce the expression of more Ia/DR antigen on the surface of macrophages as well as on other cell types. Owing at least in part to increased expression of Ia/DR antigen, IFN γ-pretreated macrophages exhibit significantly improved accessory cell functions, such as increased stimulation of mixed leukocyte reactions.

IFN γ and, to a lesser extent, IFN α and β enhance endotoxin-induced IL-1 secretion by monocytes. It has also recently been reported that incubation of *Listeria*-treated murine peritoneal macrophages with IFN γ increases the appearance of a plasma membrane form of IL-1. It is suggested that this macrophage membrane-associated IL-1 augments lymphoproliferative responses and thus promotes the capacity of accessory cells to amplify immunologic reactions.

IFN α, β, and γ have been reported to both increase and decrease synthesis and secretion of multiple proteolytic enzymes by macrophages. Synthesis of lysosomal hydrolases, esterases, and neutral proteases by macrophages endows the host with the capacity to destroy or detoxify undesirable agents. Increases in these enzymatic activities by interferon not only promote the degradative capacities of macrophages but also increase their ability to process antigens.

B. Effects of Interferon on Lymphocytes: Interferon can either augment or suppress cellular and humoral immunity, depending on the dose, time of administration, and genetic makeup of the recipient. In general, in vivo administration of interferon or interferon inducers prior to or concomitant with antigenic sensitization has considerable inhibitory effects, whereas administration of interferon subsequent to sensitization augments both cellular and humoral immune responses. The latter observation is probably of greater physiologic relevance, since IFN γ is produced relatively late in the normal course of an immune response. Opposite effects of interferon can also be demonstrated in vitro. For example, higher doses of interferon, preexposure, or simultaneous addition of interferon suppresses lymphoproliferative reactions and in vitro antibody production, whereas low doses of interferon or late addition can enhance lymphocyte proliferation and antibody production. Interferon also has both positive and negative effects on such cell-mediated immunologic phenomena as delayed hypersensitivity, graft-versus-host response, and mixed leukocyte reactions.

IFN γ plays a role in the generation of CTL activity in a mixed leukocyte reaction, since generation of CTL by alloantigen and IL-2 can be in part blocked by antiserum to IFN γ. It has recently been demonstrated that monoclonal antibodies to IFN γ prevent not only the induction of CTL activity in a mixed leukocyte reaction but also inhibit allograft rejection in vivo.

The interferons also affect cell-mediated immunity by increasing the expression of histocompatibility antigens. Interferon treatment increases the level of not only class II but also of class I histocompatibility antigens on the surface of many cell types. Increased expression of class I histocompatibility antigens makes cells more antigenic and thus better targets for CTL that specifically recognize class I antigens.

Therefore, the mechanisms by which interferons augment cellular and humoral immunity are complex and not well understood at present but are partly based on increased Ia/DR expression, increased class I antigens on CTL targets, increased IL-1 production, and directs effects on T and B cell differentiation. The immunosuppressive effects of interferon are related to its antigrowth activity or activation of T suppressor cells (or both).

C. Effects of Interferon on NK Activity: NK activity is characterized by the cytotoxic effects of LGL, in the absence of prior sensitization, against virus-infected cells, certain tumor cell lines, and normal hematopoietic cells. Both in vitro and in vivo administration of IFN α, β, or γ or interferon inducers enhances the NK activities of LGL. Recent reports indicate that IFN α has more in vitro NK-augmenting activity than IFN γ. The following mechanisms have been proposed to contribute to the increased cytocidal NK activities induced by interferon: (1) increased expression of recognition structures on LGL or target cells; (2) changes in membrane fluidity of LGL and target cells that may promote their binding capabilities; and (3) increased metabolic activity and production of cytolytic molecules by LGL. Paradoxically, pretreatment of some target cells with interferon makes them less susceptible to NK cytolysis, again

emphasizing the complexities of the actions of the interferons in the immune system.

Molecular Mechanisms of Action of Interferon

In contrast to antibodies that react with and neutralize viruses directly, the interferons do not act directly but rather establish an antiviral state and act as antiproliferative and immunomodulatory agents by inducing the synthesis of cellular proteins and by altering the metabolism of target cells. In this regard, the interferons are similar in their mechanism of action to polypeptide hormones and growth factors.

A. Interferon Receptors: The initial event in the action of the interferons is the binding to specific receptors on the cell surface. All type I interferons can bind with differing affinities to a single type of interferon receptor, whereas the receptor for type II interferon is distinct. Most cell types respond to interferon, and interferon receptors are therefore present on most cells. The binding of the interferons to their receptors is primarily of high affinity, with dissociation constants in the range of 10^{-10} to 10^{-11} mol/L. The binding is saturable, with up to 7000 type I and 13,000 type II interferon receptors per cell on some cultured cell lines. However, some cells express far fewer interferon receptors; eg, small resting T lymphocytes have only 250 IFN α and 500 IFN γ high-affinity receptors per cell.

Like other polypeptide hormones and growth factors, subsequent to the binding of the interferons to cell surface receptors, the interferon-receptor complexes cluster in coated pits and are internalized by receptor-mediated endocytosis. The interferon is then degraded in lysosomes. The interferon receptor is also internalized and inactivated after binding interferon. As a consequence, cells treated with interferon have fewer cell surface interferon receptors than untreated cells. Interferon receptors therefore undergo the ligand-induced down-regulation that occurs with receptors for many polypeptide hormones. Interferon receptor down-regulation results in a 50–80% loss of receptors; the remaining receptors can still respond to further interferon treatment.

The molecular events that transduce the signal from the cell surface interferon receptor to the rest of the cell to produce the various biologic responses to interferon are not known. Some of the biologic effects of interferon occur in the absence of interferon-receptor internalization, suggesting that a signaling mechanism must exist at the cell surface. To date, however, interferon has not been demonstrated to act via any of the signaling mechanisms that have been described for other polypeptide hormones and growth factors.

B. Interferon-Induced mRNAs and Proteins: Just as interferon types I and II have some of the same but also some distinct biologic activities, the molecular mechanisms for the action of the 2 types also have similarities and differences. Both types activate transcription of specific genes. Use of recently developed cDNA probes to interferon-induced mRNAs has demonstrated that transcription of some mRNAs is induced within 1 hour after addition of IFN α to a cell culture, but longer treatments are required with IFN γ. With IFN α, synthesis of some induced mRNAs can proceed even when protein synthesis is inhibited, whereas with IFN γ, transcription of the mRNAs requires active protein synthesis. Therefore, the induction of these mRNAs by IFN α is direct, but that by IFN γ is indirect and may first require the synthesis of an intermediate protein. These observations, combined with the existence of separate receptors for IFN α and IFN γ and the differing kinetics of action, suggest that there are different biochemical pathways for the establishment of biologic responses to types I and II interferon. Differences in the requirement for active protein synthesis between IFN α or β and IFN γ have also been observed for macrophage activation.

In Vivo Role of Interferon in Disease States

Interferon cannot normally be detected in tissues or serum, but it is rapidly produced during viral infections. Interferon also transiently appears in the serum of animals with a systemic hypersensitivity reaction following intravenous administration of large desensitizing doses of the relevant antigen.

Interferon has been detected in the serum of some patients with clinically active autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis, scleroderma, and Sjögren's syndrome. The serum interferon from SLE patients has been identified as an acid-labile form of IFN α. Interferon plays a protective role in viral diseases, since addition of interferon or interferon inducers can abrogate the development of viral diseases. This may be due in part to the pyrogenic effects of interferon and the consequent deleterious effects of fever on replication of some viruses as well as to the major antiviral action of interferon. Interferons are protective as antiviral agents even in immunodeficient subjects. Moreover, addition of antibodies to interferon exacerbates viral infections. However, the effects of interferon are not all beneficial. Although lymphocytic choriomeningitis virus (LCMV) kills adult mice, it produces a chronic disease in neonatally infected mice that culminates in chronic glomerulonephritis by 6 months of age. This is associated with high interferon levels, chronic viremia, and deposition of immune complexes containing LCMV antigens in the kidneys. Paradoxically, LCMV-infected young mice treated with anti-interferon antibodies develop less disease despite higher levels of LCMV viremia. Similarly, LCMV is more virulent for adult mouse strains that are higher interferon producers. Moreover, administration of interferon to uninfected neonatal mice results in chronic immune complex glomerulonephritis. Consequently, the interferon component of the host antiviral response, in excess, may cause aberrant autoimmune states or self-destructive host inflammatory responses.

The effectiveness of interferon as a biologic response modifier with the capacity to enhance host re-

sistance to tumors is being evaluated. Animal studies have demonstrated that interferon exerts in vivo antitumor activity. Interferon can suppress tumor growth even in nude or immunosuppressed mice. In contrast, interferon treatment is able to prevent the in vivo tumorigenicity of a cell line that is resistant to the antiproliferative action of interferon in vitro. Therefore, the antitumor activities of interferon have been attributed both to antiproliferative effects as well as to augmentation of host antitumor responses.

Clinical Experience With Interferons

IFN α has been extensively studied in phase II trials for a variety of solid tumors and hematologic malignancies. Most studies have used either highly purified lymphoblastoid-derived or recombinant IFN α preparations. Phase II clinical trials of IFN β and IFN γ are currently under way.

Although antitumor activity of IFN α for solid tumors has been quite limited, IFN α has significant antitumor activity for a variety of leukemias and lymphomas. IFN α treatment has been reported to result in an approximately 50% response rate for previously treated patients with low-grade non-Hodgkin's lymphoma. Cutaneous T cell lymphoma was also quite responsive to IFN α. Chronic myelogenous leukemia responds to IFN α with normalization of peripheral blood counts, but the Philadelphia chromosome has not been eliminated. Over 90% of hairy cell leukemia patients treated with IFN α have excellent responses, with normalization of peripheral blood counts. Encouraging results have also been reported for Kaposi's sarcoma. At present, it is difficult to explain why some malignancies respond to interferon therapy and others do not.

Patients treated with IFN α often have acute toxic reactions, including influenzalike symptoms of fever, chills, myalgias, anorexia, fatigue, headache, and occasional nausea and vomiting. Continued treatment leads to a considerable reduction in these acute symptoms. More prolonged hematologic, hepatic, gastrointestinal, and neurologic toxicities are occasionally seen.

Although IFN α has been shown to be effective with some neoplasias, the overall results of therapy with high doses of IFN α have been disappointing. However, the effects of lower immunomodulatory dose levels of the interferons, the efficacy of IFN γ, and the effects of combination therapies still need to be thoroughly explored in a variety of neoplastic and nonneoplastic conditions.

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9 Phagocytic Cells: Chemotaxis & Effector Functions of Macrophages & Granulocytes

I. MACROPHAGES*

Zena Werb, PhD

It has been a century since Elie Metchnikoff noted that during the inflammatory response leukocytes engulf microorganisms by a process he called **phagocytosis.** There are 2 types of "professional" phagocytes: the **polymorphonuclear leukocytes,** which are circulating cells that migrate into sites of inflammation (see part II of this chapter); and the **mononuclear phagocytes,** which are found circulating in the blood and fixed in tissues and also accumulate in sites of inflammation. Both of these cell types are able to recognize and ingest particles and soluble ligands through receptors on their cell surfaces and to digest these substances within lysosomal compartments. Mononuclear phagocytes, however, show much greater diversity in function and response. This diversity of structure and function is the result of progressive maturation of these cells from their bone marrow precursors, their experiences with endocytosis, and their interaction with T lymphocytes.

Since Metchnikoff coined the term "macrophage," the chief criterion for identification of these cells has been their phagocytic capacity. From functional and morphologic studies, Aschoff defined the reticuloendothelial system, which, in addition to these phagocytic histiocytes, included a variety of lymphatic and sinusoidal cells. Fibroblasts and endothelial cells, which take up colloidal gold by endocytosis, were added to the list of reticuloendothelial cells by other researchers. It is only in the last 15 years that a new classification of macrophages, monocytes, and their precursor cells has been established. On the basis of their common origin from a hematopoietic stem cell, morphologic features, and observed functions, these cells have been grouped together into one system: the mononuclear phagocyte system (Table 9–1). Although the mononuclear phagocytes can be considered to belong to one system, they display functional properties in the environment formed by other systems, including that of lymphatic organs and connective tissue.

Table 9–1. Cells of the mononuclear phagocyte system in normal and inflamed tissues.*

Cells	Localization
Stem cells (committed)	Bone marrow
↓	
Monoblasts	Bone marrow
↓	
Promonocytes	Bone marrow
↓	
Monocytes	Bone marrow
↓	
Macrophages	Tissues
Normal state, free	
Histiocytes	Connective tissues
Alveolar macrophages	Lung
Pleural and peritoneal macrophages	Serous cavities
Normal state, fixed	
Kupffer cells	Liver
Osteoclasts	Bone
Microglial cells	Nervous system
Synovial type A cells	Joints
Fixed tissue macrophages	Spleen, lymph nodes, bone marrow, and other tissues
Inflammation	
Exudate macrophages	Any tissue
Activated macrophages	Any tissue
Elicited macrophages	Any tissue
Epithelioid cells	Any tissue
Multinucleated giant cells (Langerhans types and foreign body type)	Any tissue

*Adapted from Van Furth R (editor): *Mononuclear Phagocytes: Functional Aspects.* Martinus Nijhoff, 1980.

LIFE HISTORY & TISSUE DISTRIBUTION OF MONONUCLEAR PHAGOCYTES

Mononuclear phagocytes arise in the bone marrow from a pluripotential stem cell common to all of the he-

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matopoietic cells, including erythrocytes, megakaryocytes, granulocytes, and mononuclear phagocytes. As the stem cell becomes more committed through progressive divisions, the mononuclear phagocytes and the granulocytic series continue to share a common committed stem cell. In culture, these bone marrow cells give rise to mixed colonies of granulocytes and macrophages under some conditions and monocytic colonies under others. The first progenitor cell identifiable as part of the mononuclear phagocyte system is the **monoblast** (Table 9–2), which is a round cell 10–12 μm in diameter with a small rim of basophilic cytoplasm containing a few granules. Monoblasts are capable of phagocytosis and adherence to glass; they display Fc receptors and the esterase cytochemistry typical of the more mature progeny; and they are distinct from mycloblasts, which are the precursors in the granulocytic series. In the mouse, each monoblast divides once, giving rise to the promonocytes with a cycle of about 12 hours.

The promonocytes are about 15 μm in diameter, with an indented nucleus occupying over half the cell. They share with the monoblast the typical features of mononuclear phagocytes, including prominent storage granules that stain azurophilic in smears, some of which are also positive for myeloperoxidase. The azurophil storage granules are synthesized only through this stage of maturation (Table 9–3).

The promonocytes mature into **monocytes,** which have decreased numbers of peroxidase-positive granules and an increased ratio of cytoplasm to nucleus. In contrast to the neutrophils, the marrow reserve of preformed monocytes is small. In humans, they are released into the blood within $2^1/_2$ days after their formation, where they circulate with a half-life of about 1 day, emigrating randomly from the circulating pool to the extravascular pool. In general, monocytes do not reenter the circulating pool. During inflammation, monocyte production is increased by expansion of the promonocyte pool, a decrease in cell cycle time, and release into the circulation more rapidly. **Tissue macrophages** arise by maturation of monocytes that have emigrated from the blood and by proliferation of immature macrophages in the resident macrophage population that retain their ability to respond to mitogens such as colony-stimulating factor. The relative predominance of proliferation in phagocytes versus hematopoietic origin is controversial. Most studies favor predominance of the hematopoietic route. In the normal steady state in the mouse, over half of the circulating monocytes settle in the liver as Kupffer cells, with another 15% settling in the pulmonary alveoli. The life span of the mature macrophage is probably months.

With inflammation, both the influx of blood monocytes and the local proliferation of tissue macrophages increase dramatically, and in some granulomas the turnover of the macrophages may also be increased. During inflammation, macrophages free in tissues may become activated, leading to structural and functional changes in response to mediators such as IFN γ released by antigen-stimulated lymphocytes and complement components. **Giant cells** arise either by fusion of macrophages or failure of cytokinesis during mitosis. **Epithelioid cells**—another form of mature inflammatory mononuclear phagocyte—have decreased phagocytic and digestive capacity and increased endoplasmic reticulum, which suggest that they may have secretory roles.

Macrophages are seen early in development of the lymphoid system and are known to play a role in tissue resorption associated with embryonic development. In parallel with the maturation of the lymphoid system, the mononuclear phagocytes show increasing development during fetal and neonatal life, and a number of their functions are relatively immature at birth. Replication of the tissue macrophages and their precursors appears to be under control of specific growth factors, termed colony-stimulating factors (CSF), that are produced by fibroblasts and lymphocytes. The best-char-

Table 9–2. Kinetics of mononuclear phagocytes.

Cell	Property	Human	Mouse
Monoblast	Pool size	?	2.5×10^5
	Cell cycle time	?	12 hours
Promonocyte	Pool size	6×10^8/kg	5×10^5/kg
	Cell cycle time	?	16 hours
	Percentage of nucleated marrow cells	2.9%	0.25%
Monocyte	Pool size in marrow	?	2.5×10^6
	Production rate, basal	7×10^6/h/kg	0.6×10^5/h/kg
	Production rate, inflammation	28×10^6/h/kg	1×10^5/h/kg
	Time in marrow	19–60 hours	2 hours
	Pool size in blood	2.7×10^5/mL	1×10^6/mL
	Half-time in blood	8–71 hours	22 hours
	Pool size marginating in the capillaries	3–4 times circulating pool	
Tissue macrophages			
Liver	Steady-state distribution from blood (as percentage of monocytes' steady state)	?	56
Alveoli		?	15
Peritoneum		?	8
Other		?	21
	Turnover time	?	8–60 days

Table 9–3. Changes in cellular functional characteristics with maturation of mononuclear phagocytes.

Property	Promonocyte	Monocyte	Immature Macrophage	Mature Macrophage
Proliferation	++++	+++	++	0
Azurophil granules (myeloperoxidase positive)	+++	++	±	0
Lysosomes	+	++	++++	++++
Glass adherence	+	++	+++	+++
Phagocytosis	±	+	+++	++++
Fc receptors	+	++	+++	+++
Lymphocyte interaction	?	++	++++	++++
Nonspecific esterase	+++	+++	+++	+++
Lysozyme secretion	?	++	++	++

+ = representation in the population; ± = small portion of entire population.

acterized colony-stimulating factor, CSF-1 or M-CSF, is a glycoprotein of MW 60,000 that is recognized by specific receptors, encoded by the c-*fms* proto-oncogene, found on the surfaces of mononuclear phagocytes. CSF-1 is lineage-specific for mononuclear phagocytes. Mononuclear phagocytes also proliferate and differentiate in response to CSF that affect other hematopoietic lineages. These include GM-CSF and G-CSF, which work on both myeloid and mononuclear phagocyte lineages, and interleukin-3, which also affects myeloid, erythroid, and lymphoid lineages.

ENDOCYTOSIS & MACROPHAGE PLASMA MEMBRANE RECEPTORS

The most prominent functional property of the macrophage is its ability to recognize foreign or damaged materials. **Endocytosis** by macrophages may be classified as outlined in Table 9–4. **Pinocytosis** is the ingestion of solutes from the extracellular milieu by the formation of micropinocytic vesicles, which are usually 0.2 μm in size, or by the formation of macropinocytic vesicles, which are 1–2 μm in size. Although it is likely that both types of pinocytosis are triggered by binding of a soluble ligand to specific receptors, the 2 processes are under different metabolic control, and in the case of macropinocytosis much larger samples of the solutes in the fluid phase of the extracellular medium are included in the vesicle during ingestion. Even without a phagocytic load, macrophages in culture internalize the equivalent of their entire surface area every 30 minutes. **Micropinocytosis,** which is mediated by small vesicles surrounded by a clathrin-rich bristle coat, appears to be constitutive but may be stimulated by specific ligands. **Macropinocytosis** is much more intermittent; is minimal under conditions such as culture in low concentrations of serum; and is stimulated by high concentrations of serum and by ionic compounds such as mucopolysaccharides and soluble immune complexes.

During **phagocytosis,** particles are bound to specific or nonspecific membrane receptors, then surrounded by the cell membrane, forming **phagocytic vesicles** (Fig 9–1). The binding and ingestion phases are distinct, and at low concentrations of specific ligands, particles may bind to the surface of the macrophage without being ingested. The internalization of the particles is a highly organized process that requires circumferential attachment of the macrophage receptors to the ligands attached to the particle, a process called **zippering.** If the ligands are not present at sufficient density for this recognition phenomenon to occur or if the receptors on the macrophage surface are not sufficiently mobile in the fluid phase of the membrane, this process stops at binding. Binding of soluble and insoluble materials to macrophages—and subsequent ingestion of these materials by pinocytosis or phagocytosis—is ascribed to a rapidly increasing number of specific binding activities of the macrophage. While the existence of specific receptors has yet to be demonstrated formally in certain cases, the macrophage of plasma membrane has receptors for a large variety of immune and nonimmune molecules (Table 9–5).

Receptors that bind the Fc portion of immunoglobulins and the C3 components of complement endow the macrophage with its capacity to recognize opsonized particles. There are at least 4 classes of **Fc receptors.** A proteinase-sensitive receptor is able to bind monomers or complexes of IgG of certain subclasses (in humans, IgG1 and IgG3; in the mouse, IgG2a; in the guinea pig, IgG2). Antibodies binding to this receptor are cytophilic, ie, they bind to the macrophages first and then interact with antigen. There is a proteinase-resistant Fc receptor that binds to and mediates endocytosis of antigen-antibody complexes or aggregates of IgG subclasses (IgG2 and IgG4 in humans and IgG1 and IgG2b in the mouse) (Fig 9–2). A third type of proteinase-resistant Fc receptor specific for IgG3 has been demonstrated in the mouse. Macrophages also have a receptor that specifically binds IgE. During phagocytosis mediated by these Fc receptors, the receptors are cleared from the membrane, gradually returning over a subsequent period of 6–24 hours. The number or activity of Fc re-

Table 9–4. Types of endocytosis by macrophages.

Type	Vesicle Size	Special Requirements
Pinocytosis		
Micropinocytosis	0.2 μm	Receptor-mediated; does not require protein synthesis
Macropinocytosis	1–2 μm	Receptor-triggered (?); requires protein synthesis
Phagocytosis		
Immune phagocytosis	> 0.4 μm	Mediated by Fc or complement receptors
Nonimmune phagocytosis	> 0.4 μm	Mediated by other specific or nonspecific receptors

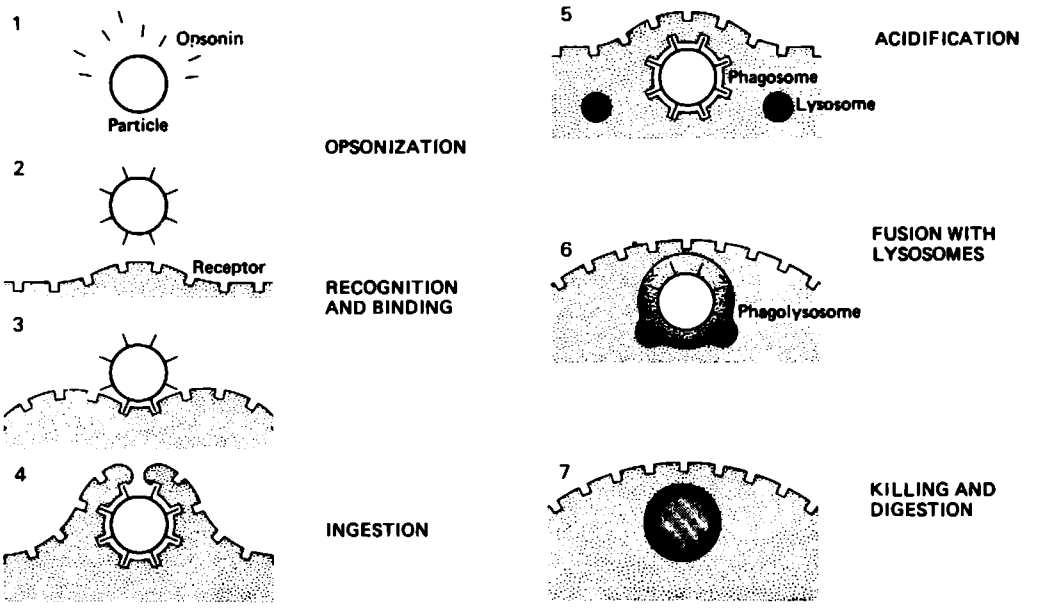

Figure 9–1. Events in phagocytosis by phagocytes. (*I*) Particles such as microorganisms and tumor cells can be recognized by the phagocyte only if coated by a molecule for which the phagocyte has a receptor. Substances that make the particle attractive for ingestion have been termed opsonins and include immunoglobulins, complement, fragments, and fibronectin. Once coated with the opsonin (*II*), the opsonized particle can then be recognized and can bind to specific receptors on the phagocyte surface (*III*). In the case of some effectors, the binding event does not necessarily lead to subsequent engulfment by the phagocyte. For ingestion to occur, the particle is circumferentially surrounded by the membrane of the phagocyte during sequential zippering between the recognized opsonin on the particle surface and the receptors on the phagocyte surface (*IV*). The receptors may need to move in the fluid bilayer of the phagocyte membrane in order to make contact with the ligand. Once the circumferential zippering is complete, a membrane-bounded vesicle separated from the plasma membrane (a phagosome) forms (*V*). At some time during the period of formation of the phagosome, a number of the receptors and plasma membrane proteins present on the phagosomal membrane are recycled back to the plasma membrane. The phagosomes then fuse with existing primary and secondary lysosomes to form a phagolysosome (*VI*), in which the organisms may be killed, with subsequent digestion at acid pH (*VII*).

ceptors is modified by inflammation and certain disease states, when the number of Fc receptors may quadruple.

The **receptors for complement** are independent of the Fc receptors. In unstimulated macrophages or monocytes, the C3 receptors are much more efficient at mediating binding than at mediating ingestion. It is likely that in vivo, the C3 receptors and Fc receptors function synergistically. With macrophage activation, the complement receptors acquire the ability to mediate ingestion on their own. There are at least 3 complement receptors on mononuclear phagocytes of humans, guinea pigs, and mice. Complement receptor 1 is specific for C3b and complement receptor 2 for C3d. C5a is chemotactic for mononuclear phagocytes, and it is likely that this third complement receptor is expressed on these cells as well as on granulocytes.

Macrophages also have **receptors for lymphokines,** which are involved in macrophage activation; and for colony-stimulating factors, which regulate macrophage proliferation. Receptors for insulin have also been demonstrated on macrophages. Macrophages also have receptors recognizing complex carbohydrates and fucosyl and mannosyl terminal glycoproteins. These receptors may be important to the clearing of glycoproteins and in the recognition of senescent cells, heterologous erythrocytes, yeasts and other fungi, bacteria, and parasites. Macrophages recognize α_2-macroglobulin-proteinase complexes, which may be important in in vivo clearance of enzymes such as thrombin, plasmin, kallikrein, and activated complement components. Receptors for proteins containing iron may play a role in the secretion of iron by macrophages. Receptors for fibrin/fibrinogen complexes may play an important role in clearance of fibrin from the circulation or inflammatory sites. The receptor for fibronectin may aid in the adhesion of monocytes to areas containing breaches in the integrity of the endothelial lining of the vessels, and fibronectin may also act as an opsonin for certain particles. Macrophages may play an important role in regulation of triglyceride and cholesterol metabolism through their receptors for normal and altered lipoproteins.

Once formed at the periphery of the cell, pinocytic and phagocytic vesicles flow into the macrophage toward the perinuclear area, guided by microtubules, in an energy-requiring process that utilizes the abundant

Table 9–5. Surface receptors of mononuclear phagocytes.

Immune receptors
Fc domain of immunoglobulins
IgG monomers (proteinase-sensitive receptor)
IgG complexes (proteinase-insensitive receptors)
IgE
Complement
C3b, C3bi
C3d
Lymphokines (migration inhibitory factor)
[illegible] receptors
Hormone receptors
Colony-stimulating factor
Insulin
Thrombin
[illegible] or fucosyl terminal (yeast cell walls)
[illegible]-proteinase complexes
[illegible]
[illegible]
[illegible] fibrinogen
[illegible]
[illegible] receptors
Anionic low-density lipoproteins (eg, acetylated)
Apolipoproteins B and E (chylomicron remnants, β-migrating, very low density lipoproteins, low-density lipoproteins)

amount of cytoplasmic contractile proteins. In the perinuclear area, the endocytic vacuoles become secondary lysosomes after fusion with primary lysosomes. Alternatively, the endocytic vacuoles may fuse with preexisting secondary lysosomes. At some stage between the initial formation of the endocytic vesicle membrane and the formation of the secondary lysosome membrane, the contents become acidified and portions of the membrane and its plasma membrane receptors and some contents are recycled back to the cell surface. Within the lysosomal compartment, the phagocytized and pinocytized contents—as well as some of the plasma membrane proteins—are digested at acid pH by more than 40 hydrolytic enzymes within the lysosomes (Table 9–6). Bacterial macromolecules

Figure 9–2. Scanning electron micrograph of a macrophage in the process of ingesting, by its Fc receptors, IgG-coated sheep erythrocytes. (Courtesy of R Takeya.)

Table 9–6. Lysosomal acid hydrolases of macrophages.

Enzyme	Major Substrates
Phosphatases	Phosphate esters
Aryl sulfatase	Sulfate esters
Cholesteryl esterase	Cholesteryl esters of lipoproteins
Triglyceride lipase	Triglycerides of lipoproteins
Phospholipases	Phospholipids of bacteria, lipoproteins
Glycosidases	Carbohydrates, glycosaminoglycans, etc
Proteinases	
Cathepsin B	Collagen, proteoglycans
Cathepsin D	Hemoglobin

such as proteins, complex carbohydrates, and lipids are digested to subunits of MW 200 or less that can escape from the lysosome into the cytoplasm.

Chronic intracellular pathogens have evolved a variety of mechanisms designed to subvert the processes of phagocytosis, fusion of phagosomes with lysosomes, and function of lysosomal enzymes. *Trypanosoma cruzi* and certain enveloped viruses penetrate the phagosomal membrane and escape into the cytoplasm. *Mycobacterium tuberculosis* and *Toxoplasma* somehow prevent fusion of lysosomes with phagosomes, a process that can be mimicked by a variety of polyanions, and with lectins such as Con A. *Mycobacterium lepraemurium* is surrounded by a cell wall that is resistant to hydrolysis.

CHEMOTAXIS

Mononuclear phagocytes have the capacity to migrate into and through tissues. This migration may be random or specifically directed toward an inflammatory chemical stimulus, a process called chemotaxis. How mononuclear phagocytes find or make a path through the connective tissue is not known. However, macrophages contain on their surfaces—and secrete—proteolytic enzymes active at tissue pH that may be important in their ability to migrate in vivo. Numerous substances generated during inflammation have a capacity to enhance the speed of macrophage movement (chemokinesis) and to orient the movement in the direction of an increased concentration gradient of the agent (chemotaxis). Substances chemotactic for macrophages include factors derived from serum, particularly the C5a anaphylatoxin. C5a is released as a consequence of activation of complement by antibody-antigen complexes, by bacteria, by the classic or alternative pathways, or by the direct action of cell-derived proteolytic enzymes on C5. Other chemotactic substances include bacterial products such as N-formylmethionyl peptides and products from stimulated B and T lymphocytes that attract mononuclear phagocytes to sites of inflammation and delayed hypersensitivity reactions. Factors produced by fibroblasts, fragments of collagen, elastin, and denatured proteins may help attract macrophages to sites of tissue injury. Also important in the consideration of attraction of macrophages to sites of inflammation are substances

that inhibit the random migration of macrophages and thus prevent migration away from sites of inflammation. Two chemically distinct classes of substances seem to function in the retention of macrophages: lymphokines (macrophage migration inhibitory factor, macrophage activation factor) and proteolytic enzymes produced during activation of complement (factor Bb) and of the fibrinolytic system (plasmin).

Abnormalities of chemotactic responsiveness of monocytes and macrophages have been noted in individuals with tumors, certain defects in immunity (eg, Wiskott-Aldrich syndrome), and recurrent candidiasis and after infection of macrophages with certain viruses such as influenza and herpes simplex viruses.

METABOLISM DURING PHAGOCYTOSIS

The ingestion of particles by macrophages as well as by neutrophils (see part II of this chapter) is accompanied by a **respiratory burst** observed as a dramatic increase in the consumption of oxygen and activation of a membrane-associated oxidase that is dependent on reduced nicotinamide adenine dinucleotide phosphate (NADPH). This oxidase reduces molecular oxygen to superoxide anion, which in turn dismutates to hydrogen peroxide (Table 9–7). **Superoxide** and **hydrogen peroxide** can interact to give rise to **hydroxyl radical** and, possibly, **singlet oxygen.** Some of the hydrogen peroxide is destroyed by glutathione peroxidase, with the oxidation of reduced glutathione. Reduced glutathione is regenerated by glutathione reductase and is accompanied by the oxidation of reduced NADPH. This NADPH is derived from the hexose monophosphate shunt, which is also stimulated to accommodate the increased utilization of oxygen. The reactive metabolites of oxygen that are generated at or near the cell surface and within the phagocytic vacuole exert antimicrobial and antitumor cell effects. The macrophage itself is protected from the noxious effects of these oxygen metabolites by its glutathione peroxidase and catalase. However, there is evidence that these oxygen metabolites may produce host damage, particularly in lung and synovium.

The respiratory burst, although intimately connected with phagocytosis, is not an essential accompaniment to phagocytosis. Recent evidence suggests that free tissue macrophages and newly recruited monocytes—but not fixed tissue macrophages—can respond to lymphokines and phagocytic stimuli by mounting a respiratory burst. The failure of fixed tissue macrophages, such as Kupffer cells, to produce active metabolites of oxygen may be important in protecting tissues from damage during the scavenger functions of the macrophage. Many soluble agents, including antigen-antibody complexes, C5a, ionophores, and tumor promoters, can trigger the respiratory burst without phagocytosis. The respiratory burst can also be triggered by opsonized particles or surfaces when phagocytosis is frustrated by the use of a drug such as cytochalasin B. Phagocytosis can also proceed without the respiratory burst. In particular, phagocytosis mediated by complement C3 or C3bi receptors does not trigger release of hydrogen peroxide or arachidonic acid metabolites. In chronic granulomatous disease, in which an enzyme required for the respiratory burst is missing, phagocytosis occurs normally. In addition to the reactive species of oxygen, such as superoxide anion ($O_2^{-}\bullet$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\bullet$), and singlet oxygen ($^1O_2$), there are a number of other potential microbicidal mechanisms in macrophages. The antimicrobial effects of hydrogen peroxide are augmented by halide ions in the presence of myeloperoxidase (which is found in granules of monocytes but not mature macrophages) or of catalase (which is found in mature macrophages) by the generation of hypochlorite ions. Other microbicidal mechanisms include hydrogen ion, lysosome, complement components, and lysosomal hydrolases. The relative importance of these mechanisms varies with different microbes. Macrophages may also inhibit the growth of or kill tumor cells by several types of interaction: antibody-dependent cell-mediated cytotoxicity, in which the tumor cells have been coated with antibodies directed to the cell surface; cytostasis, produced by thymidine or by the depletion of arginine by arginase; and mechanisms dependent on the release of reactive species of oxygen from macrophages.

Table 9–7. Events of the respiratory burst associated with phagocytosis.

Activation of the hexose monophosphate shunt and the glutathione reductase - glutathione peroxidase cycle
Activation of a membrane-associated NADPH oxidase, with increased consumption of oxygen and generation of superoxide anion and hydrogen peroxidase
Formation of hydroxyl radical, singlet oxygen, and other metabolites of reactive oxygen
Chemiluminescence

MACROPHAGE SECRETION

Phagocytosis by macrophages has been studied for over a century, but it is only in the last decade that the importance of macrophages as secretory cells has been recognized. Over 50 secretion products of macrophages have been identified (Table 9–8). The role of macrophages as secretory cells may be as important in their interaction with the extracellular milieu as is their role as phagocytic cells. Some of the secretion products of the macrophage influence the inflammatory process at its many steps. This secretion by macrophages is under complex control and varies with the status of the mononuclear phagocytes. Lysosome and complement components appear to be secreted constitutively by macrophages in all states of stimulation. The secretion of other products such as arachidonic metabolites, acid hydrolases, and neutral proteinases

Table 9–8. Secreted products of macrophages.

- **Enzymes**
  - Neutral proteinases
    - Plasminogen activator
    - Metal-dependent elastase
    - Collagenase, specific for interstitial collagens (types I, II, III)
    - Collagenase, specific for basement membrane collagen (type IV)
    - Collagenase (gelatinase), specific for pericellular collagen (type V)
    - Stromelysin
    - Cytolytic proteinase
  - Arginase
  - Lysozyme
  - Lipoprotein lipase
  - Angiotensin-converting enzyme
  - Acid hydrolases
    - Proteinases and peptidases
    - Glycosidases
    - Phosphatases
    - Lipases
    - Others
- **Plasma proteins**
  - α_2-Macroglobulin
  - α_1-Proteinase inhibitor
  - Tissue inhibitor of metalloproteinases
  - Fibronectin
  - Transcobalamin II
  - Apolipoprotein E
  - Coagulation proteins
    - Tissue thromboplastin
    - Factor V
    - Factor VII
    - Factor IX
    - Factor X
  - Complement components
    - C1
    - C2
    - C3
    - C4
    - C5
- **Plasma proteins (cont'd)**
  - Complement components (cont'd)
    - Properdin
    - Factor B
    - Factor D
    - Factor I (C3b inactivator)
    - Factor H (β_1 H, C3b inactivator accelerator)
- **Reactive metabolites of oxygen**
  - Superoxide anion
  - Hydrogen peroxide
  - Others
- **Bioactive lipids**
  - Prostaglandin E_2
  - 6-Ketoprostaglandin $F_{1\alpha}$
  - Thromboxane B_2
  - Leukotriene C (slow-reacting substance of anaphylaxis)
  - 12-Hydroxyeicosatetranoic acid
  - Others
- **Nucleotide metabolites**
  - cAMP
  - Thymidine
  - Uracil
  - Uric acid
- **Factors regulating cellular functions**
  - Interleukin-1 (endogenous pyrogen)
  - Angiogenesis factor
  - Interferon
  - Factors promoting proliferation of—
    - Fibroblasts
    - Endothelial cells
    - T cells
    - B cells
    - Myeloid cell precursors (GM-CSF)
  - Factors inhibiting proliferation of—
    - Tumor cells
    - *Listeria monocytogenes*
  - Erythropoietin

is triggered and regulated by engagement of specific receptors, by endocytosis, or by exposure of macrophages to membrane-active drugs, including tumor promoters, ionophores, and endotoxin. Secretion can also be regulated by activated lymphocytes, by tissue pH and oxygen tension, and by other factors influencing the inflammatory activity of macrophages.

Macrophages secrete a number of enzymes active at neutral and acid pH. **Plasminogen activator** activates the plasma zymogen plasminogen to plasmin, which is of particular interest because of the ability of plasmin to lyse fibrin, activate C1 and C3, and cleave activated Hageman factor into components that convert prekallikrein to kallikrein. Macrophages stimulated by inflammation or activated by endotoxin or infection secrete considerably more plasminogen activator than do resident macrophages, and its secretion is further amplified by phagocytosis of poorly digested materials. **Elastases, collagenases,** and **gelatinases,** secreted by macrophages, may be important in the degradation of connective tissue macromolecules such as elastin, collagen, and proteoglycans and may promote the migration of macrophages through basement membranes. Elastase also degrades immunoglobulins, α_1-proteinase inhibitor, fibronectin, and fibrinogen. These enzymes may be present in granulomas and at the site of delayed hypersensitivity reactions and may explain some of the degradation seen in these areas. Lysozyme is able to degrade the cell wall polysaccharide of certain organisms and, in concert with lysosomal hydrolases, which are also released from cells, can contribute to bacterial and tissue breakdown in inflammatory lesions where there is a sufficiently low pH. **Arginase,** an enzyme that degrades arginine, is secreted by inflammatory exudative cells. Because arginine is required for normal metabolism of many cells, its depletion may lead to inhibition of cellular function or cytostasis for tumor cells. Lipoprotein lipase may have an important function in the metabolism of lipoproteins by macrophages.

Macrophages also secrete a variety of plasma proteins. Many of these proteins have previously been identified as secretion products of hepatocytes. Alpha$_2$-macroglobulin is a prominent inhibitor of all

known proteolytic enzymes and thus may regulate the potential of the macrophages to lyse connective tissue macromolecules and participate in the complement, coagulation, and kinin cascades. It is interesting to note that macrophages contain on their surface a receptor for α_2-macroglobulin-proteinase complexes. Alpha$_1$-proteinase inhibitor—an inhibitor of granulocyte elastase and other serine proteinases—and the tissue inhibitor of metalloproteinases are also products of mononuclear phagocytes. **Fibronectin,** a protein which is opsonic for particles coated with denatured collagen (gelatin) and which also has a structural role in connective tissue, is secreted in large quantities by the macrophage. Macrophages are able to utilize fibronectin as opsonin and for adhesion to a variety of cells. Macrophages also secrete transcobalamin II, which is involved in the transport of vitamin B_{12}, and apolipoprotein E, a protein with dual functions in lipid transport and immunoregulation. Macrophages have on their surface a receptor that allows them to recognize and ingest certain lipoproteins containing apolipoprotein E (eg, β-migrating very low density lipoproteins and chylomicron remnants). Macrophages also produce proteins involved in the coagulation cascade: tissue thromboplastin and factors V, VII, IX, and X. Because the macrophage also participates in the fibrinolytic system, it has a dual role in clot formation and lysis. Complement components regulate the chemotactic and acidic metabolic and secretory activity of the macrophage; in turn, the macrophage also engages in the synthesis and secretion of complement components. The macrophage synthesizes and secretes virtually all components of the classic and alternative pathways of complement and thus provides additional direct links between acute and chronic hypersensitivity responses.

Nonprotein substances of pharmacologic potency secreted by macrophages certainly play a role in inflammation as important as those of the protein components. The production of reactive metabolites of oxygen have already been commented on. These products are potent oxidizing agents that may inactivate thiol groups of proteins, break bonds in proteins, oxidize lipids and nucleic acids, and initiate free radical chain reactions that may contribute significantly to the tissue damage accompanying inflammation. Macrophages have been shown to produce large amounts of prostaglandin E_2. Prostaglandins affect the functions of macrophages as well as of other cells, including suppression of B cell proliferation, suppression of myeloid stem cell proliferation, and inhibition of mitogen responsiveness. They have also been implicated as the important element in inducing acute-phase reactants in plasma during inflammation. One of the bioactive lipids, leukotriene C, has been identified as the slow-reacting substance of anaphylaxis. Thus, the macrophage may participate in the acute as well as in the chronic inflammatory response.

The macrophage also produces factors, mostly protein in nature, that regulate the functions of other cells. Interleukin-1, which has also been called lymphocyte-activating factor and endogenous pyrogen, is secreted by macrophages. This protein induces T cells to produce interleukin-2, induces fibroblasts to secrete collagenase and prostaglandin E, induces acute-phase protein synthesis by hepatocytes, and binds to receptors in the hypothalamus, which are then signaled to reset the body temperature to a higher level. During inflammation or hypoxia, macrophages secrete **angiogenesis factor,** which promotes the neovascularization of tissues. They also secrete factors that promote the proliferation of fibroblasts, endothelial cells, and myeloid precursors. Factors including **interferon** inhibit the proliferation of cells, including tumor cells, and proliferation of bacteria. Within the broad spectrum of their states of activity, macrophages may promote or inhibit the same processes and contribute to many facets of the inflammatory response and the regulation of cellular functions.

PROPERTIES OF INFLAMMATORY & ACTIVATED MACROPHAGES

Almost every structural and functional feature of the macrophage has been reported to change when the cells or the host animal are treated in some way. Mackaness demonstrated that lymphocytes recognizing a microbial antigen induced in macrophages an enhanced antimicrobial activity against the specific immunizing pathogen as well as against unrelated pathogens. These cells were observed to spread faster on glass, ruffled their membranes more prominently, contained many lysosomes, and were more phagocytic. These macrophages have been termed **activated.** Macrophages from animals treated with nonmicrobial inflammatory stimuli display a similar but not identical pattern of changes (Table 9–9). They show many of the same metabolic, phagocytic, plasma membrane enzyme, and lysosomal changes as activated macrophages, but they lack enhanced antimicrobial and antitumor activity. It is now evident that these macrophages should not be referred to as activated but as inflammatory or elicited. Activation of macrophages occurs as a result of their interaction with mediators from antigen- or mitogen-stimulated lymphocytes, termed lymphokines, or with the products of activation of complement components, or with IFN γ (Fig 9–3). Activation of macrophages may also occur by direct pharmacologic action of agents such as endotoxin. In contrast to the specific immunologic activation of T and B cells, the activation of macrophages is not specific to the primary infecting organism. For example, activated macrophages from animals infected with *Trichinella* or *Toxoplasma* are cytotoxic to tumor cells and to unrelated intracellular and extracellular pathogens such as *Listeria* and *Trypanosoma*.

Induction and functional expression of macrophage activation in vivo depend on a complex series of events, and the macrophage is capable of undergoing or expressing a large number of functional and

Table 9–9. Activity of inflammatory macrophages compared to that of quiescent resident macrophages.

Nonspecific inflammatory events
Increased size
Increased rate of spreading
Increased adherence to glass
Increased rate and extent of phagocytosis
IgG-coated particles
C3b-coated particles
Modification of plasma membrane ectoenzymes
Decreased 5'-nucleotidase
Increased alkaline phosphodiesterase
Increased rate of fluid phase pinocytosis
Secreted proteins
Increased plasminogen activator
Increased elastase
Increased collagenase
Increased cellular ATP
Increased O_2 consumption, glucose, O_2^- release
Increased prostaglandin release
Decreased leukotriene C production
Lymphokine-mediated events
Increased H_2O_2 release
Increased microbicidal activity
Increased tumor cytostasis and killing
Decreased secretion of apolipoprotein E and other proteins

metabolic alterations. The enhancement of microbicidal activity depends on sensitized T cells, which may be native or adoptively transferred in vivo. In culture, unstimulated macrophages may be activated by providing either living sensitized T cells and their antigens or the lymphocyte-derived products from the interaction of viable sensitized T cells and antigen. The substrate for the activation process includes the unstimulated free macrophages resident in the tissue spaces as well as newly recruited and mature monocytes migrating from the vascular pool during activation by the immunologically mediated specific event. Although some of the properties of fixed tissue macrophages, such as Kupffer cells, are altered in response to inflammatory stimuli, these cells are defective in mounting a respiratory burst and in generating reactive metabolites of oxygen—in contrast to free tissue macrophages and newly emigrated monocytes. The properties of the activated macrophages may be understood without necessarily proceeding step-by-step through the sequence of properties developed during the nonspecific inflammatory response. The number of steps in the activation process and the mechanism by which it is regulated are not fully worked out, and the precise biochemical and metabolic processes that define the activated state are still controversial.

THE ROLE OF MACROPHAGES IN REGULATING THE IMMUNE RESPONSE

Macrophages play an important role in initiation and regulation of the immune response, both in vivo and in culture. The macrophage may participate in the immune response in 2 ways. There may be nonspecific roles that depend on the capacity of macrophages to improve the viability of lymphocytes—an action that can be mimicked by the addition of 2-mercaptoethanol in culture. Alternatively, macrophages may suppress the proliferation of lymphocytes nonspecifically through thymidine, arginase, complement cleavage products, prostaglandin E, and interferon. Macrophages also may alter the function of lymphocytes more specifically. They produce interleukin-1 (formerly called leukocyte-activating factor), which specifically alters T cell function as described in Chapter 7. Another function of macrophages is in the processing and presentation of immunologically active molecules to the lymphocyte. This function requires that

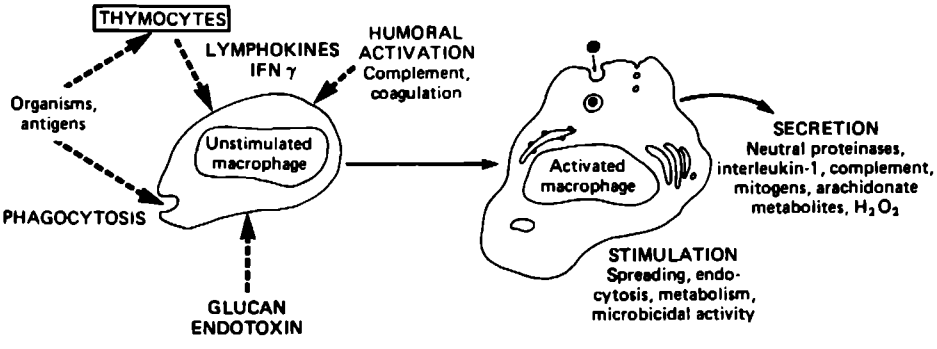

Figure 9–3. Schematic representation of the process of activation of unstimulated macrophages. Macrophages might be activated by interaction with organisms and antigens directly, by interaction with activated T lymphocytes, by interaction with the product of the activation of complement and coagulation systems, or by certain chemicals such as glucan and phorbol diesters (phorbol myristate acetate, PMA). These result in the activation of the macrophage, which is able to phagocytize more readily; secretes neutral proteinases, interleukin-1, and complement components at a greater rate; and is stimulated to spread and undergo other metabolic activities.

the T cell and macrophage display the same major histocompatibility-encoded determinants (Ia antigens) (Fig 9–4). Not all macrophages express Ia antigen; only a subpopulation of monocytes and macrophages from specifically activated populations are positive for this marker.

DISORDERS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

A variety of human diseases are associated with increased numbers of monocytes and tissue macrophages or with abnormalities in the functions of monocytes and macrophages (Table 9–10). For example, mononuclear phagocytes may proliferate appropriately in response to *M tuberculosis*, producing a monocytosis or reactive hyperplasia manifested by enlargement of organs rich in these cells, such as the spleen and lymph nodes. On the other hand, they may proliferate inappropriately to far exceed normal levels, as in monocytic leukemia or other malignant histiocytic proliferative diseases. Another group of disorders are those in which increased numbers of lysosomes are seen in tissue macrophages or when material taken up by phagocytosis accumulates intracellularly more rapidly than it can be disposed of by metabolic processes. These can be called storage diseases, the result of ingestion of a nondigestible substance, an overloading of iron as in hemosiderosis, or inborn errors of metabolism in which a specific genetic defect of macrophage enzyme function has occurred (eg, Gaucher's disease, Hurler's disease). There is also a group of macrophage dysfunction syndromes. These include genetic abnormalities such as chronic granulomatous disease, in which both macrophages and polymorphonuclear leukocytes lack an enzyme important in the respiratory burst seen in phagocytosis and do not have the ability to kill pathogens susceptible to reactive metabolites of oxygen. Defects in synthesis and secretion of complement components produce macrophage dysfunction because macrophages

Table 9–10. Participation of mononuclear phagocytes in pathologic processes.

Process	Examples
Inflammatory processes	
Acute inflammation	Monocytosis
	Infectious and parasitic diseases
Chronic inflammation	Reactive hyperplasia
	Infectious and parasitic diseases
Chronic granulomatous inflammation	Reactive hyperplasia
	Intracellular parasites
	Beryllium toxicity
	Sarcoidosis
Destructive granulomas	Wegener's granulomatosis
	Midline granuloma
Storage diseases	Enzyme deficiencies
	Mucolipopolysaccharidoses
	Hurler's disease
	Lipidoses
	Tay-Sachs disease
	Nondigestible substances
	Hemosiderosis
	Xanthomas
	Pneumoconiosis
Mononuclear phagocyte dysfunctions	Chronic granulomatous disease
	Osteopetrosis
	Complement deficiencies
Neoplastic processes	Monocytic leukemia
	Malignant histiocytosis
	Histiocytosis X
	Histiocytic medullary reticulosis

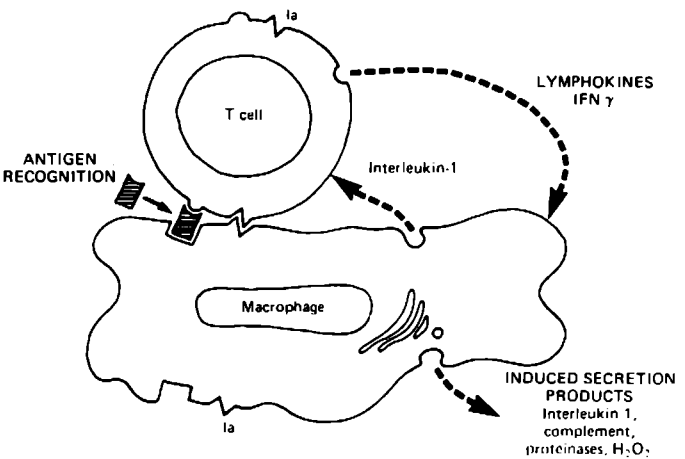

Figure 9–4. Diagrammatic representation of the interaction between macrophages and T cells that is dependent on the compatibility of products (Ia antigens) of genes in the major histocompatibility complex. The interaction depends on recognition of conventional antigens as well as Ia determinants and results in the production of specific mediators capable of activating macrophages.

Table 9–11. Functions of macrophages in vivo.

Host defense against microorganisms and tumor cells
Participation in acute inflammatory response
Participation in chronic inflammatory response
Production of endogenous pyrogen (IL-1)
Immune phagocytosis
Microbial and cytostatic activities
Accessory and regulatory cell for T cells, B cells, and natural killer cells
Secretion of complement components, fibronectin
Wound healing
Regulation of coagulation and fibrinolysis
Tissue debridement by phagocytosis and secreted enzymes
Regulation of neovascularization
Regulation of endothelial cells and fibroblasts
Bone resorption
Removal of dead cells, inhaled particles, and effete erythrocytes
Lipid metabolism
Removal of chylomicron remnants and altered lipoproteins
Secretion of apolipoprotein E
Regulation of hematopoiesis
Direct cell-cell interaction with myeloid and erythroid precursors
Production of colony-stimulating factors and erythropoietin

are a prominent source of these complement components, and the products of complement activation are important for macrophage, phagocytic, and migratory functions. In osteopetrosis, abnormally low numbers of osteoclasts are found in bone, and bone resorption is abnormal. In addition to these disorders, high concentrations of glucocorticosteroids and ionizing radiation may interfere with the macrophage defense system, including macrophage migration into tissues and macrophage proliferation; thus, opportunistic infections may occur frequently. Another class of macrophage dysfunction has been described in animals in which there are genetically determined differences in macrophage responses to lipopolysaccharides. It is likely that there is heterogeneity in lipopolysaccharide responsiveness in humans as well.

The mononuclear phagocyte system has many functions. It participates in host defense, tissue hygiene, wound healing, and general homeostatic mechanisms, interacting with many other cells and tissue fluids throughout the body (Table 9–11). The chameleonlike adaptations of the macrophages to their tissue environments endow these cells with the ability to influence many processes negatively, positively, or in complex feedback loops. Our challenge is to unravel and reconstruct these mechanisms, so that we can understand how these events are controlled in vivo.

II. GRANULOCYTES

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A common feature of most forms of immunologically induced acute inflammation is the accumulation of polymorphonuclear leukocytes (**neutrophils**) at the site of the reaction. These cells are not merely innocent bystanders to events that occur at a focus of inflammation but play an active role in the mediation of these events. Indeed, it has been established that acute inflammation in virtually all multicellular organisms occurs largely as a consequence of a coordinated series of events whereby phagocytic cells attempt to defend the host from "foreign invaders."

In humans as in most higher organisms, the neutrophil is primarily responsible for maintaining normal host defenses against invading microorganisms. Neutrophils are suitably equipped to seek out, ingest, and destroy most "foreign invaders." This requires a series of discrete but coordinated steps which include adherence to endothelium, extravascular emigration, directed migration toward particles to be ingested (**chemotaxis**), membrane recognition of (and attachment to) particles, engulfment of particles (**phagocytosis**), fusion of lysosomes with—and discharge of lysosomal constituents into—phagocytic vacuoles (**degranulation**), and a burst of oxidative metabolism (with generation of oxygen-derived free radicals and hydrogen peroxide).

In the sections that follow, each of these functions of neutrophils will be discussed in some detail. Emphasis will be placed on recent developments in immunology, cell biology, and pathology that have helped to elucidate mechanisms whereby neutrophils promote inflammation and kill microorganisms.

ADHERENCE OF NEUTROPHILS TO VASCULAR ENDOTHELIUM & EXTRAVASCULAR EMIGRATION

The total pool of peripheral blood neutrophils is composed of 2 readily exchangeable subpools. One of these subpools is composed of neutrophils that circulate in the central axial stream within blood vessels. This "circulating pool" of cells represents about half of the total blood neutrophils in humans and is readily measured by the standard clinical leukocyte count and differential. Cells in the "marginal pool," representing the remainder of total blood neutrophils, circulate out of the axial stream and move slowly along the vascular endothelium. Under normal physiologic conditions, neutrophils in the marginal pool adhere to endothelial surfaces only rarely and momentarily.

Regardless of the cause, one of the earliest events accompanying acute inflammation is an increase in the margination of circulating neutrophils (ie, adherence of these cells to vascular endothelium). Adherence is a prerequisite for subsequent diapedesis into the extravascular compartment. Adherence of neutrophils to endothelial cells without subsequent diapedesis and emigration may be the only manifestation of inflammation after minimal damage to tissues.

A number of diverse substances with chemotactic activity for neutrophils (eg, synthetic and complement-derived peptides) have been found capable of

Table 9–12. Factors that influence neutrophil adhesiveness.

Augmenting Factors	Inhibitory Factors
Plasma from patients with diverse forms of inflammation	Plasma from patients receiving anti-inflammatory drugs
cGMP	cAMP
Propranolol	Epinephrine
Deuterium oxide	Colchicine
Chemotactic factors	Ethanol
Divalent cations	Local anesthetics

augmenting the adhesiveness of these cells. This is manifested in vitro by increased adherence of neutrophils to substrates such as glass, plastic, nylon fibers, and cultured endothelial cells as well as by aggregation of neutrophils in suspension. Administration of chemotactic factors intravenously to experimental animals results in prompt neutropenia, markedly increased margination of neutrophils, and pulmonary vascular leukostasis. Thus, naturally occurring chemoattractants are not only responsible for directed extravascular migration of neutrophils but also play a role in promoting localized adherence of neutrophils to vascular endothelium (Table 9–12).

The precise mechanism by which high concentrations of chemotactic factors augment the adhesiveness of neutrophils is unknown. Whereas altered surface charge may be an important factor, roles for neutrophil granule constituents, oxygenation products of arachidonic acid, and plasma membrane proteins also have to be considered. Concerning the latter, evidence has been presented that chemotactic factors augment adherence of neutrophils to diverse surfaces by provoking translocation of "adhesive glycoproteins" from an intracellular pool (perhaps in specific granules) to the cell surface.

DIRECTED MIGRATION OF NEUTROPHILS: CHEMOTAXIS

Motile cells such as neutrophils are capable of migrating in a directed fashion along gradients of chemical stimuli. Extravascular emigration of neutrophils most likely is caused by gradients of such chemical attractants that are established between inflamed tissues and blood vessels. There is now abundant evidence that directed migration (chemotaxis) of neutrophils is important both in the mediation of inflammation and in the maintenance of normal host defenses against infection. Directed migration of neutrophils in humans is mediated largely by fluid phase components of the complement system (eg, C5a and C5a des Arg) which are generated by cleavage of native complement proteins as a consequence of activation of either the classic or alternative pathways. Other chemoattractants for neutrophils include products of bacteria (eg, N-formyl methionyl peptides), products of coagulation and fibrinolysis, oxidized lipids (eg, leukotriene B_4), and products of stimulated leukocytes (see below). The precise mechanisms whereby these factors are recognized by neutrophils and thus become capable of initiating directed motility are largely unknown. Only very recently has evidence been obtained that these factors interact with and influence neutrophil membranes. Indeed, structurally specific receptors for C5a, N-formyl methionyl peptides, leukotrienes, and leukocyte-derived chemotactic factors have been demonstrated on the surfaces of both human and rabbit neutrophils.

Chemotactic factors have been shown to be capable of provoking other membrane-dependent neutrophil responses. For example, they stimulate neutrophil oxidative metabolism and provoke selective discharge from neutrophils of lysosomal constituents (Table 9–13). These actions of chemotactic factors are discussed in sections that follow.

CELL-DERIVED CHEMOTACTIC FACTORS

Chemotactic factors other than those derived from either complement activation or bacteria are important for recruiting neutrophils to sites of inflammation. These other chemotactic factors are derived either directly from cells or indirectly by the action of cellular enzymes on extracellular substrates. Alveolar macrophages, for example, secrete both polypeptides and lipids that are chemotactic for neutrophils. Neutrophils themselves synthesize and release a low-molecular-weight chemotactically active peptide when exposed to monosodium urate crystals, calcium pyrophosphate crystals, and diamond crystals. This peptide, and perhaps similar material released from neutrophils exposed to immune complexes, may amplify some acute inflammatory reactions.

Another way in which neutrophils can amplify acute inflammatory reactions is by releasing granule constitutents that promote the formation of complement (C5)-derived peptides. For example, proteinases (primarily elastase and cathepsin G) released from neutrophils during phagocytosis cleave native human C5 at neutral pH to yield chemotactically active fragments. It should be emphasized, however, that whereas this activity can be demonstrated easily with isolated C5, it cannot be demonstrated in whole serum unless the enzymes are added in excess of the inhibitory capacity of α_1-proteinase inhibitor and α_2-macroglobulin. Consequently, except in patients with

Table 9–13. Chemotactic factor–induced responses of neutrophils.

Directed migration (chemotaxis).
Increased adhesiveness.
Decreased net negative surface charge.
Translocation of monovalent (Na^+ and K^+) and divalent (Ca^{2+}) cations.
Increased oxidative metabolism (generation of superoxide anion radicals).
Degranulation (lysosomal enzyme release).

hereditary deficiency of α_1-proteinase inhibitor, it is not clear whether neutrophil lysosomal proteinases cleave native C5 in vivo.

Human neutrophils exposed to phagocytizable stimuli also release a substance that activates the alternative complement pathway in whole human serum to yield biologically active C5-derived peptides. This high-molecular-weight substance is heat-stable (60 °C for 30 minutes) and is localized to neutrophil-specific granules. Thus, human neutrophils contain materials that interact with the complement system in at least 2 ways to generate biologically active C5-derived peptides.

Although products of arachidonic acid formed by the cyclooxygenase pathway influence the ability of neutrophils to accumulate at sites of inflammation (by virtue of their effects on vascular tone and vascular permeability), only products formed by lipoxygenases exhibit potent chemotactic activity. For example, 12-hydroxyeicosatetraenoic acid (12-HETE) (formed in platelets) and 5-hydroxyeicosatetraenoic acid (5-HETE) (formed in leukocytes) are chemotactic for human neutrophils. At very low concentrations, 12-HETE and 5-HETE exhibit chemokinetic properties; ie, they enhance random locomotion of neutrophils. The most potent of the chemotactic factors that can be produced from arachidonic acid is 5,12-dihydroxyeicosatetraenoic acid (leukotriene B_4).

Leukotriene B_4 provokes directed migration of human neutrophils in vitro at concentrations less than 10 ng/mL. In fact, leukotriene B_4 is as potent a chemoattractant for human neutrophils as the complement-derived peptide C5a. In vivo, leukotriene B_4 induces adherence of neutrophils to the walls of postcapillary venules, neutrophil-dependent increases in vascular permeability, and accumulation of neutrophils in skin and lung.

Another product of arachidonic acid that possesses potent chemotactic activity for human neutrophils is 5,15-dihydroxyeicosatetraenoic acid (8,15-diHETE). Formed by the 15-lipoxygenase pathway (primarily in eosinophils), 8,15-diHETE is nearly as potent as leukotriene B_4 with respect to its chemotactic activity for human neutrophils. It also resembles leukotriene B_4 with respect to its ability to stimulate random migration of neutrophils.

Finally, a great deal of attention has been directed recently at the role played by another chemotactic lipid in mediating acute inflammation. Appropriately stimulated neutrophils, eosinophils, monocytes, alveolar macrophages, and endothelial cells synthesize and release a biologically active lipid that is similar to, or identical with, 1-0-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine. Because of its ability to aggregate rabbit platelets, this lipid has been referred to as platelet-activating factor. It is now recognized, however, that platelet-activating factor also is a potent chemotactic factor for neutrophils. In vivo, platelet-activating factor increases extravascular permeability and provokes migration of neutrophils from blood vessels into tissue.

RECOGNITION: NEUTROPHIL CELL SURFACE RECEPTOR FUNCTION

There are 2 major constituents of serum (**opsonins**) that act upon certain bacteria, fungi, and other particles to increase their "palatability." One is heat-stable (at 56 °C for 30 minutes) and is found chiefly in serum from animals previously exposed to the test particle (immune serum). The other is heat-labile and is present in fresh normal serum. The heat-stable constituent is recognized now as immunoglobulin (antibody) of the IgG class (in particular, subclasses IgG1 and IgG3). These molecules—or, more specifically, their Fc portions—are recognized by phagocytic cells by means of what appear to be rather specific **receptors** ("Fc receptors" or "IgG receptors"). It has been estimated that 75–90% of human peripheral blood neutrophils bear receptors on their surfaces for IgG-coated particles.

Binding to neutrophils of antigen-antibody complexes, immunoglobulin-coated particles, and aggregated immunoglobulins depends upon the integrity of the Fc regions of the immunoglobulin molecules. Binding to neutrophils of either aggregated or antigen-complexed immunoglobulins exceeds that of the corresponding monomeric immunoglobulin molecules. At least 2 hypotheses have been offered to account for this observation. One hypothesis assumes that although monomeric immunoglobulins have an exposed binding site on their Fc regions available for attachment to neutrophils, such binding is unstable. More stable—and therefore detectable—binding results only from the formation of complexes with multiple sites. Thus, stable binding of polyvalent antigen-antibody complexes or of immunoglobulin aggregates would result from cooperative binding by Fc receptors of several molecules (which exponentially increases binding of the whole complex). The alternative hypothesis suggests that either aggregation of immunoglobulins or interactions between antibodies and antigens produce allosteric changes in the conformation of immunoglobulin molecules, thereby exposing sites capable of interacting with the neutrophil surface. Thus, the tertiary structure of intact immunoglobulins may influence binding to neutrophils.

Structurally specific receptors for the Fc regions of IgG immunoglobulins have been identified on the surface of neutrophils. When examined indirectly by fluorescence microscopy and by electron microscopy, Fc receptors appear to be mobile in the plane of the neutrophil plasma membrane. A close correlation has been observed between Fc receptor redistribution and immune complex-induced neutrophil responses, suggesting a relationship between activation of neutrophils and surface receptor mobility.

Heat-labile opsonic activity is attributable principally to a fragment of the third component of complement. Cleavage of C3, as a consequence of activation of either the classic or alternative complement pathways, yields 2 fragments, C3a and C3b. The larger of

these fragments, C3b, is capable in its nascent state of becoming fixed to the surfaces of cells and other particles (including bacteria and fungi). C3b renders the particles to which it is attached recognizable by phagocytic leukocytes (including neutrophils) and mediates firm particle-cell adherence by interacting with "C3 receptors." In humans, receptors for fragments of C3 can be demonstrated on over 90% of neutrophils in peripheral blood.

Human neutrophils possess 2 classes of C3 receptors: those that recognize C3b (complement receptor 1, or CR1) and those that recognize iC3b (CR3). Only C3b receptors (CR1), however, have been isolated from neutrophil plasma membranes and characterized biochemically. Human neutrophils do not possess receptors for C3d (CR2). There is indirect evidence that optimal expression of C3b receptor activity on the surface of neutrophils is dependent upon the mobility of these receptors in the plane of the lipid bilayer. It has been suggested that aggregation of C3b receptors is a prerequisite for C3-dependent cytoadherence.

It should not be concluded from the foregoing discussion that neutrophils recognize only particles or surfaces coated with fragments of C3 or IgG. Nor should it be concluded that recognition is mediated only by structurally specific surface membrane receptors. Indeed, the term receptor may signify no more than an ability of neutrophils to recognize a given molecule and to be activated by it. Whereas molecular entities within (or on) the neutrophil surface membrane are presumed to mediate these functions, the presence of some of them can only be inferred. Receptors for some ligands have been neither identified nor isolated from human neutrophils. Consequently, it is best to consider neutrophil surface membrane receptors as "recognition units" that may or may not be represented by specific single molecules or even by complex intramembranous structures. Recognition units, of necessity, would be linked to "effector units" (of similarly vague composition) that trigger or initiate specific cellular functions.

PHAGOCYTOSIS

When contact is established between a neutrophil and a suitable particle, the particle is ingested by the cell, a process termed phagocytosis. Direct observations of phagocytosis by light microscopy and electron microscopy have revealed that attachment of neutrophils to a suitable small particle results in the formation at the site of attachment of pseudopodia that surround the particle and ultimately fuse at its distal pole. The process of particle engulfment by neutrophils requires energy (supplied by anaerobic glycolysis) as well as complex interactions between cytoplasmic contractile proteins (ie, actin and myosin) and the plasma membrane.

Whereas neutrophils appear capable of ingesting seemingly "inert" particles (eg, polystyrene latex beads), particularly under conditions where particle-cell contact is maximized, opsonins such as C3b or IgG clearly increase the rate and extent of particle uptake. Whether they do so merely by promoting particle-cell contact or whether they are indeed capable of activating ingestion is controversial. Several investigators, using erythrocytes coated with IgG or C3b (or both), have demonstrated that the neutrophil C3b receptor is involved chiefly in recognition and attachment and only inefficiently promotes ingestion of bound or adherent particles. In contrast, particle binding to the neutrophil IgG receptor, while less efficient, appears necessary for the induction of optimal phagocytosis. Thus, C3b and IgG have separate but synergistic roles in phagocytosis (Table 9–14). Depending upon the experimental conditions, the presence of particle-bound C3b is able to reduce 100-fold the amount of IgG required to promote engulfment of particles. Under certain conditions, C3b receptors may serve to overcome electrostatic repulsion and permit contact between the neutrophil surface and moieties on particles that promote engulfment. The role of C3b in opsonization is mainly one of establishing contact between particle and phagocyte.

Neutrophils are capable of ingesting certain particles in the complete absence of complement or immunoglobulins. It is likely that such particles have chemical moieties on their surfaces that not only permit attachment by neutrophils but also behave as "surrogate immunoglobulins" and promote ingestion. If this is the case, particles that require only C3b on their surfaces for optimal ingestion by neutrophils might be expected to be ingestible in the native state if particle-cell contact is enhanced. Indeed, such a phenomenon has been demonstrated.

DEGRANULATION

As indicated in the previous sections, phagocytosis by neutrophils requires recognition of the particle to be ingested, adherence or binding of the particle to the cell surface, and, finally, engulfment of the particle within a vacuole and closure of the plasma membrane. Shortly after (or coincident with) these events, lysosomal granules fuse with those portions of the plasma membrane that constitute the phagocytic vacuole. Membrane fusion leads to the discharge of lysosomal enzymes and other granule constituents into the newly

Table 9–14. Roles of IgG and C3b in adherence and phagocytosis.*

	Adherence	Phagocytosis
EA (IgM)†	–	–
EA (IgG)	+	+
EA (IgM-C3b)	+++	–
EA (IgG-C3b)	+++	+++

*Adapted from Ehlenberger AG, Nussenzweig V: *J Exp Med* 1977;145:357.

†Sheep erythrocytes (E) sensitized with IgM or IgG antibodies (A).

formed—or forming—phagosome (degranulation). The resultant structure has been termed a **phagolysosome.** Phagolysosome formation underlies many normal cellular functions and is crucial for normal host defenses (see Chapter 13). Degranulation does not appear to be a uniform process. Primary (azurophil) and secondary (specific) granules of neutrophils discharge their contents at different rates during phagocytosis. Secondary granules appear to fuse first with the phagosome membrane.

Under certain conditions, lysosomal enzymes may be released to the outside of the cell. One mechanism whereby lysosomal constituents are extruded from neutrophils is simply "cell death." When neutrophils are exposed to a variety of toxins, injury to the plasma membrane is an early consequence, and all intracellular materials are released from the injured cell, including those ordinarily sequestered with lysosomes. Biologic detergents, for example, act in this manner to cause primary lysis of the cell membrane and, subsequently, disruption of lysosomes.

Under some circumstances, materials gain access to the interior of the cell's vacuolar system, wherein they cause membranes of lysosomes to rupture. Such damage leads to release of cytoplasmic enzymes and other intracellular constituents as the cell dies by a kind of "perforation from within" of its vacuolar system. Crystalline substances such as monosodium urate and silica act on phagocytic cells in this fashion.

Another mechanism of enzyme release from neutrophils involves the discharge of lysosomal hydrolases into the surrounding medium of intact cells engaging in endocytosis. Such release is not accompanied by leakage of cytoplasmic enzymes and appears to be due to the extrusion of lysosomal materials from incompletely closed phagosomes, open at their external borders to the extracellular space but already joined at their internal borders by lysosomes actively discharging their contents into the vacuole. The cell engaging in phagocytosis remains viable, but its released lysosomal contents are free to act upon surrounding tissues. This probably is a common mechanism of tissue injury in a variety of disease states.

Phagocytosis per se is not an absolute prerequisite for degranulation of neutrophils. There is substantial evidence that degranulation of neutrophils can be provoked not only by appropriate ligand-surface membrane receptor interactions but also by "nonspecific" membrane perturbation. For example, when neutrophils encounter immune complexes or aggregated immunoglobulins deposited upon solid surfaces, such as Millipore filters or collagen membranes, the cells adhere to these surfaces and selectively release their lysosomal constituents. A similar phenomenon may occur when adherent cells encounter some soluble stimuli such as the complement component C5a. Enzyme release under these conditions occurs by a process called **reverse endocytosis** during which merger of granules with the plasma membrane results in discharge of lysosomal constituents directly to the outside of the cell as though into a phagocytic vacuole. Phagocytosis does not occur, and the viability of adherent cells is not altered. This mechanism of lysosomal enzyme release from neutrophils very likely is pertinent to the pathogenesis of tissue injury in several diseases in which immune complexes are deposited upon cell surfaces or extracellular structures such as vascular basement membranes.

NEUTROPHIL OXIDATIVE METABOLISM

The process of ingestion by neutrophils of suitably opsonized microorganisms is necessary, but usually not sufficient, for optimal killing. Whereas the acidic environment within phagocytic vacuoles can limit the growth of some bacteria, effective killing requires products of molecular oxygen or granule constituents (ie, enzymes or nonenzymatic substances). Stimulated neutrophils consume large amounts of molecular oxygen. This "respiratory burst" leads to the production of superoxide anion radicals and hydrogen peroxide as well as stimulation of the hexose monophosphate shunt pathway of glucose oxidation. Simultaneously, cytoplasmic granules (ie, lysosomes) fuse with invaginated portions of the plasma membrane. Products of the respiratory burst and granule constituents act together within the confines of phagocytic vacuoles to kill and degrade ingested microorganisms. Under some circumstances, however, products of the respiratory burst and granule constituents are released from neutrophils and act extracellularly to promote inflammation and tissue injury.

The bulk of oxygen that is consumed by stimulated neutrophils is reduced to water through the formation of several highly reactive intermediates. Oxygen, in its ground state, contains 2 unpaired electrons with parallel spins. Since these present a "barrier" to the insertion of additional pairs of electrons, ground state oxygen normally is reduced by univalent, or single electron steps. When oxygen accepts a single electron, it is converted to the superoxide anion radical ($O_2^{-\bullet}$) (equation 1), which can act both as a reductant and as an oxidant:

$$O_2 + e^- \rightarrow O_2^{-\bullet} \qquad \ldots(1)$$

In human neutrophils, this reaction is catalyzed by a pyridine nucleotide-dependent oxidase. The bulk of oxygen that is consumed by stimulated neutrophils is converted directly to superoxide anion radicals. In fact, metabolically "activated" neutrophils appear to be the principal physiologic sources of superoxide anion radicals in extracellular fluids. The increased ability of stimulated neutrophils to reduce nitroblue tetrazolium dye is a reflection of enhanced generation of superoxide anion radicals. In addition to superoxide, neutrophils generate hydrogen peroxide, hydroxyl radicals, and, perhaps, singlet oxygen.

Hydrogen peroxide may be formed either directly from oxygen by divalent reduction or indirectly by

univalent reduction of superoxide, ie, by dismutation (equation 2):

$$O_2^{-}\bullet + O_2^{-}\bullet + 2H^{+} \rightarrow H_2O_2 + O_2 \quad \ldots(2)$$

Dismutation can occur spontaneously or may be catalyzed by the enzyme superoxide dismutase. Spontaneous dismutation, although rapid, does not preclude participation by superoxide in reactions that yield other radical species.

Further reduction of hydrogen peroxide yields hydroxyl radicals (OH•), extremely potent oxidants that can react with a variety of organic substrates. A mechanism that has been proposed for the generation of hydroxyl radicals involves reduction of hydrogen peroxide by superoxide anion radicals in the presence of ferrous ions (equation 3):

$$H_2O_2 + O_2^{-}\bullet \rightarrow O_2 + OH\bullet + OH^{-} \quad \ldots(3)$$

Another potentially toxic product of oxygen metabolism by activated neutrophils is singlet oxygen ($^1O_2$). Ground state oxygen has 2 electrons in separate orbitals with their angular momentum opposed but with parallel spins. The first electronically excited state, or singlet oxygen, has both electrons in a single orbital in which the angular momentum is the same. Singlet oxygen may be formed by spontaneous dismutation of superoxide anion radicals, by reactions involving superoxide and hydroxyl radicals, or by reactions involving superoxide and hydrogen peroxide.

As is the case with degranulation, the metabolic events described above can be stimulated in the absence of phagocytosis. For example, neutrophils adherent to certain nonphagocytizable surfaces have been observed to increase their oxidative metabolism, particularly if the surfaces are coated with immune complexes or aggregated IgG. Similarly, neutrophils exposed to certain soluble stimuli have been demonstrated to increase their oxygen uptake, production of superoxide anion, hexose monophosphate shunt activity, and NBT reduction. These soluble stimuli include immune reactants, such as aggregated IgG and complement-derived chemotactic peptides, as well as a variety of nonimmune, surface-reactive compounds such as phospholipase C, phorbol myristate acetate, Con A, and digitonin. The metabolic responses of neutrophils to these soluble stimuli require intact viable cells and closely resemble those observed during phagocytosis.

It appears that cell surface stimulation of neutrophils is sufficient, in the absence of phagocytosis, to provoke the degranulation and the burst of oxidative metabolism that ordinarily accompany ingestion of particles. What is the relation between these 2 responses of neutrophils to stimulation? Whereas it is true that the 2 responses appear to be inseparable when normal neutrophils are allowed to ingest particles, studies with nonphagocytic stimuli indicate that the 2 phenomena can occur independently. For example, it has been demonstrated that there is no significant correlation between the ability of various stimuli to provoke lysosomal enzyme release from neutrophils (degranulation) and their ability to enhance generation of superoxide anion.

Although products of the respiratory burst exhibit some microbicidal activity, they are utilized most effectively by neutrophils in conjunction with peroxidases. Myeloperoxidase, for example, is a lysosomal enzyme (in human neutrophils and monocytes) that catalyzes hydrogen peroxide-mediated oxidation of halide ions to hypohalite ions (equation 4):

$$X^{-} + H_2O_2 \rightarrow XO^{-} + H_2O \quad \ldots(4)$$

One product of this myeloperoxidase-catalyzed reaction is hypochlorite, an extremely potent microbicidal agent. Chloramines, formed by reactions between hypochlorite and either ammonia or amines, also are potent microbicidal agents. Finally, other microbicidal oxidants (eg, hydroxyl radicals, singlet oxygen) may be generated as by-products of reactions involving myeloperoxidase. Although it is not entirely clear how products of the myeloperoxidase-hydrogen peroxide-halide system alter the viability of bacteria and fungi, it is well established that this system participates in oxygen-dependent killing by neutrophils.

As indicated above, products of the respiratory burst and granule enzymes (eg, myeloperoxidase) are not always confined within phagocytic vacuoles. Under a variety of circumstances, potent microbicidal substances may be released from neutrophils and may act extracellularly to damage adjacent tissues.

A role for products of the respiratory burst and peroxidases in the production of injury to cells has been demonstrated in several experimental systems. Targets have included tumor cells, human erythrocytes, human neutrophils, and cultured human endothelial cells. Although the precise nature of the reactive molecules that mediate cytotoxicity is unknown, results of most studies indicate roles for hypochlorite, chloramines, hydroxyl radicals, and singlet oxygen. These reactive species can oxidize sulfhydryls, thioethers, pyridine nucleotides, and unsaturated fatty acids. In addition, they can directly alter deoxyribose moieties of DNA.

Another way in which products of the respiratory burst and peroxidases may provoke tissue injury is by altering the function of naturally occurring antiproteinases. Oxidants produced by stimulated human neutrophils, for example, are capable of inactivating α_1-proteinase inhibitor. Inactivation of the antielastase activity of the inhibitor is dependent upon release from stimulated cells of hydrogen peroxide, superoxide anion radicals, and myeloperoxidase. In the absence of stimulated neutrophils, α_1-proteinase inhibitor (but not α_2-macroglobulin or α_1-antichymotrypsin) can be inactivated by superoxide-generating systems as well as by purified myeloperoxidase in the presence of hydrogen peroxide and halide ions. Inactivation is due to oxidation of methionine thioether residues to sulfoxides at or near the active site of the proteinase inhibitor. Oxidized α_1-proteinase inhibitor is incapable

of forming stable complexes with elastase and, consequently, of influencing the elastolytic activity of the enzyme. It has been suggested that oxidants released from stimulated neutrophils at sites of either acute or chronic inflammation could alter the local balance between proteinases released by these cells and naturally occurring antiproteinases. Such an imbalance would render adjacent tissues or other macromolecules more susceptible to proteolytic attack.

OTHER MICROBICIDAL SYSTEMS OF NEUTROPHILS

Whereas the myeloperoxidase-hydrogen peroxide-halide system clearly is microbicidal, neutrophils are able to kill some microorganisms effectively by other pathways. For example, normal neutrophils are quite capable of killing some microorganisms under strictly anaerobic conditions. Furthermore, patients with hereditary myeloperoxidase deficiency are not unusually susceptible to bacterial or fungal infections.

Contributing to the oxygen- and myeloperoxidase-independent microbicidal activity of neutrophils are a variety of proteins that are stored in cytoplasmic granules. Lactoferrin, for example, is a constituent of human neutrophil-specific granules that limits proliferation of bacteria by virtue of its ability to chelate iron, an essential growth factor for many microbial species. Lysozyme, found in both specific and azurophil granules of human neutrophils, is an enzyme that efficiently hydrolyzes the cell walls of *Micrococcus lysodeikticus*. Elastase, cathepsin G, and other cationic proteins found in human neutrophil azurophil granules also have been found capable of killing some bacterial species.

One granule constitutent, termed the **bactericidal/permeability-increasing protein (BPI)**, has been studied extensively. BPI from human neutrophils is a highly cationic protein with a molecular weight of 59,000. BPI binds specifically to the outer membrane of some gram-negative bacteria and very rapidly increases the permeability of this structure. Although the inner membrane remains intact and synthesis of macromolecules continues unaltered for at least 30–60 minutes, bacteria exposed to BPI for only a few seconds irreversibly lose their ability to replicate. Rough strains of bacteria, with surface structures that are more anionic and hydrophobic than those found on corresponding smooth strains, bind BPI more avidly and are more sensitive to the microbicidal actions of the protein. Of the multiple components of the bacterial outer membrane, lipopolysaccharides appear to be major determinants of BPI action. Only lipopolysaccharide-containing bacteria are sensitive to BPI, and varied sensitivity among strains of some bacteria is due primarily to differences in polysaccharide chain length.

Although the precise mechanism whereby BPI kills bacteria is unknown, there is ample evidence that this protein is an important microbicidal component of neutrophils. For example, the rate and extent of killing of some bacteria by intact neutrophils is remarkably similar in disrupted cells, crude extracts, and highly purified fractions containing comparable amounts of BPI. Additional evidence that BPI is a major antimicrobial component of neutrophils was provided by studies comparing killing of bacteria by cells under aerobic and anaerobic conditions. Both *Salmonella typhimurium* MR-10 and *S typhimurium* MS were ingested and killed effectively in the absence and presence of oxygen. In contrast, *Staphylococcus epidermidis* was effectively killed only in the presence of oxygen. Thus, killing of some bacteria by neutrophils does not require a respiratory burst.

In contrast to BPI, which does not alter the viability of gram-positive bacteria or fungi, other cationic proteins in neutrophils exhibit broad antimicrobial activity. Human and rabbit neutrophils, for example, contain a "family" of low-molecular-weight cationic peptides that kill *Candida* species as well as a wide variety of gram-positive and gram-negative bacteria.

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10 The Complement System

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MECHANISM OF ACTION OF THE COMPLEMENT SYSTEM

The complement (C) system is the primary humoral mediator of antigen-antibody reactions. It consists of at least 20 chemically and immunologically distinct plasma proteins capable of interacting with one another, with antibody, and with cell membranes. Following activation of the system, these interactions lead to the generation of biologic activity which ranges from lysis of a spectrum of different kinds of cells, bacteria, and viruses to direct mediation of inflammatory processes. In addition, complement is able to recruit and enlist the participation of other humoral and cellular effector systems and induce histamine release from mast cells, directed migration of leukocytes, phagocytosis, and release of lysosomal constituents from phagocytes.

The individual proteins of this system are normally present in the circulation as functionally inactive molecules. Together they make up approximately 15% (w/w) of the plasma globulin fraction. The native precursor molecules are designated by numerals—C1, C2, C3, etc—or, in the case of certain of the components, by symbols or trivial names—properdin, factor B, factor D, etc. Each complement component must be activated sequentially under appropriate conditions in order for a complement reaction to progress. Thus, activation is not a single event but rather a dynamic process that enables the proteins to become interacting members of a functionally integrated system. Complement enzymes formed during the activation process are designated by a bar placed over the symbol of the component, eg, $\overline{C1s}$, factor $\overline{B}$. An activated, biologically active but nonenzymatic state of a component may also be identified by a bar placed over the term for the component, eg, $\overline{C5b67}$. Fragments of the components arising from enzymatic cleavage are denoted by letters following the term employed for the component, eg, C4a, C4b.

There are 2 parallel but entirely independent mechanisms or pathways leading to activation of the terminal, biologically important portion of the complement sequence (Fig 10–1). These mechanisms of activation, termed the classic and the alternative or properdin pathways, respectively, are triggered by different substances. Each involves several reaction steps. The 2 activation pathways converge at the midpoint of the complement system, and the remainder of the reaction sequence, involving the reactions of C5 through C9, is common to both pathways. The terminal portion of the complement sequence may also be directly activated by certain noncomplement serum and cellular enzymes without participation of the early reacting factors. Among the trypsinlike enzymes capable of activating at the C3 or C5 stage of the reaction are the fibrinolytic enzyme plasmin and certain lysosomal enzymes (Fig 10–1).

THE CLASSIC COMPLEMENT PATHWAY

The classic pathway may be activated by antigen-antibody complexes or aggregated immunoglobulins (Table 10–1). Human immunoglobulins belonging to the IgG1, IgG2, and IgG3 subclasses and IgM class are capable of initiating the classic pathway, whereas the IgG4 subclass and IgA, IgD, and IgE classes are inactive in this regard. Among the IgG subclasses, IgG3 is most active, followed (in order) by IgG1 and IgG2. Immunologic activation occurs via binding of the first complement component (C1) to a site located in the Fc region of the IgG or IgM molecule.

The classic pathway may also be activated nonimmunologically by a number of chemically diverse substances, including DNA, C-reactive protein, and certain cellular membranes and trypsinlike enzymes (Table 10–1). Activation occurs by direct binding of C1 to these substances or, in the case of enzymes such as the fibrinolytic enzyme plasmin, by direct proteolytic attack on the C1 molecule.

The classic pathway comprises the reaction steps of the first (C1), second (C2), third (C3), and fourth (C4) complement components. The pathway may be subdivided into 2 functional units: first, activation of the first component, C1; and second, generation of 2 re-

Table 10–1. Activation of the complement (C) system.

	Classic	Alternative
Immunologic	IgG, IgM	
Nonimmunologic	Trypsinlike enzymes	Trypsinlike enzymes
	DNA	Lipopolysaccharides
	Staphylococcal protein A	Plant and bacterial polysaccharides
	C-reactive protein	Cobra venom factor

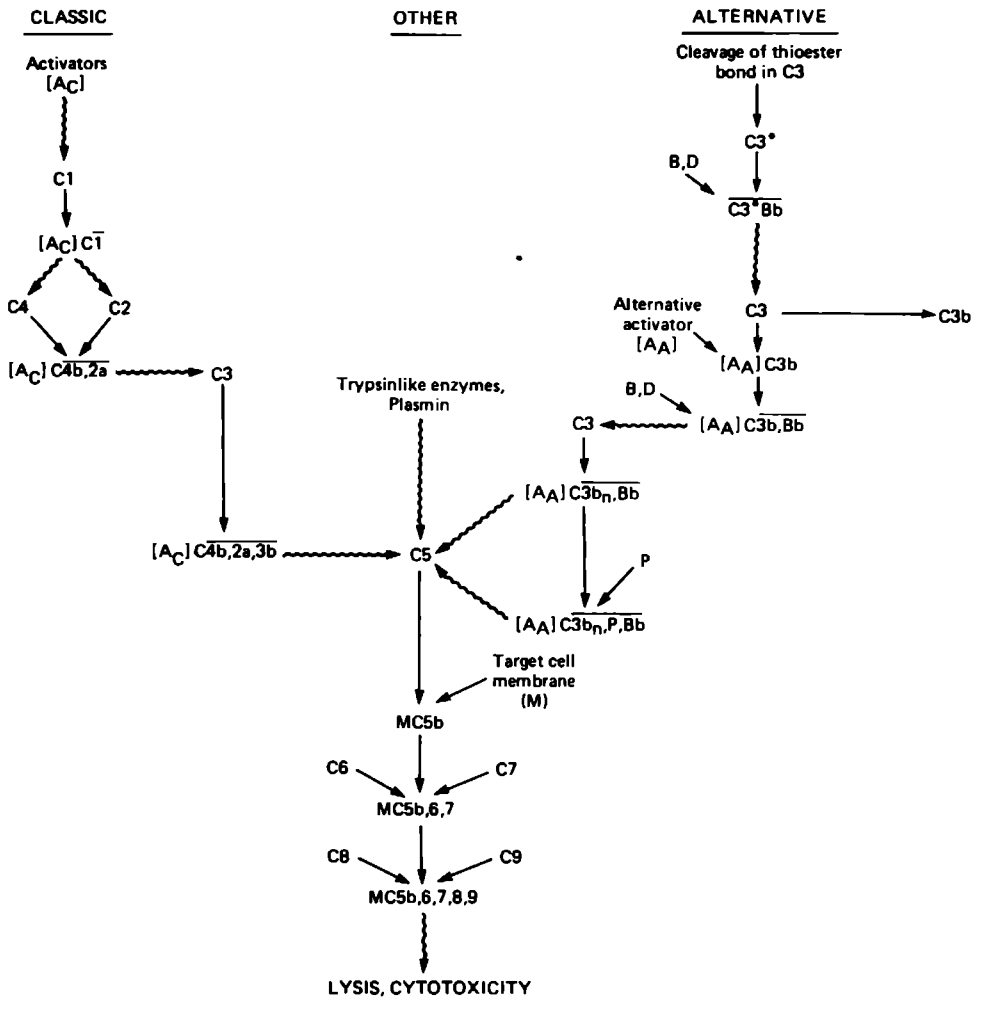

Figure 10–1. Schematic diagram of the mechanisms of assembly of the complement (C) system on the surface of a complement activator. (P= properdin; B = factor B; D = factor D.)

lated complex complement enzymes, $\overline{C4b2a}$ and $\overline{C4b2a3b}$.

C1

The steps involved in the activation of C1 following attachment to the activating agent or after proteolytic attack make up the first functional unit of the classic complement pathway. C1 consists of 3 distinct protein molecules—termed C1q, C1r, and C1s—which are held together by a calcium-dependent bond (Table 10–2). C1 is present in the circulation as a firm C1q-C1r-C1s macromolecule, and individual subunits are found only in pathologic conditions. C1 may, however, be readily dissociated and reassociated on removal and restoration of calcium ions. C1q has a molecular weight of 400,000; electrophoretically, it is one of the most cationic proteins of human serum. It is a unique serum protein in that its structure is chemically very similar to that of collagen or basement membrane. Like collagen, it contains large amounts of glycine and the hydroxylated amino acids hydroxylysine and hydroxyproline and a significant carbohydrate content consisting in large part of glucose and galactose residues linked as disaccharide units to the hydroxyl group of hydroxylysine. C1q contains a total of 18 polypeptide chains of 3 distinct types that are organized into a structure, visualized by electron microscopy, consisting of 6 peripheral globular portions connected by fibrillar strands to a central structure. The polypeptide chains of the collagenlike portion of each of the 6 subunits form, in all likelihood, a triple helix. A schematic representation of the C1q molecule is shown in Fig 10–2. The C1q molecule bears the sites which enable the C1 molecule to bind to the Fc region of IgG and IgM molecules and is able to bind approximately 6 IgG molecules. The IgG or IgM bind-

Table 10–2. Properties of the complement components and complement regulators.

Name	Synonyms	Molecular Weight	Electrophoretic Mobility	Approximate Plasma Concentration (μg/mL)
Classic pathway				
C1q	C'O, 11S protein	400,000	γ_2	70
C1r	...	190,000	β	34
C1s	$\overline{C1}$ esterase	87,000	α	31
C2	...	117,000	β_1	25
C3	β_1C	185,000	β_1	1600
C4	β_1E	206,000	β_1	600
Alternative pathway				
C3	Factor A, hydrazine-sensitive factor (HSF)	185,000	β_1	1300
Factor B	C3 proactivator (C3PA), glycine-rich β glycoprotein (GBG), β_2-glycoprotein II	93,000	β_2	200
Factor D	C3 proactivator convertase (C3PAse), glycine-rich β glycoproteinase (GBG)	24,000	α	1
Factor I	C3b inactivator, KAF, C3b INA	88,000	β	34
Factor H	β_1H, C3b inactivator accelerator	150,000	β_1	500
Properdin	...	220,000	γ_2	25
Membrane attack mechanism				
C5	β_1F	191,000	β_1	70
C6	...	120,000	β_2	64
C7	...	110,000	β_2	56
C8	...	151,000	γ_1	55
C9	...	71,000	α	59
Complement regulators				
C1 inhibitor	$\overline{C1}$ esterase inhibitor, $\overline{C1}$ inactivator	105,000	α_2	180
C4 binding protein	C4bp	>500,000	β	...
Factor I	C3b inactivator, KAF, C3b INA	88,000	β	34
Factor H	β_1H, C3b inactivator accelerator	150,000	β_1	500
Properdin	...	220,000	γ_2	25
S protein	Membrane attack complex inhibitor, MAC INH	80,000	α	505
Anaphylatoxin inactivator	A1, SCPB, carboxypeptidase N	300,000	α	...
Abnormal proteins				
C3 nephritic factor	C3 NeF, NF	160,000	γ	...

ing sites appear to be associated with the peripheral globular subunits of the C1q molecule.

C1r is a β-globulin with a molecular weight of 190,000 (Table 10–2). Following attachment of C1 via C1q to activators, thus forming [Ac]C1, C1r acquires the ability to enzymatically activate C1s. Integrity of the C1 macromolecule and calcium ions are required for this process.

C1s is an α-globulin with a molecular weight of 87,000 (Table 10–2). Following cleavage of a single peptide bond in the C1s molecule by C1r, C1s acquires proteolytic enzyme activity. The de novo generated enzyme is of the serine esterase type and thus inhibitable by analogs of diisopropylfluorophosphate. The enzymatic site is located in the smaller of the 2 chains produced on proteolytic activation of the molecules; these chains are linked to each other by disulfide bridges. Upon generation of the $\overline{C1s}$ enzyme, the initial phase of the classic complement pathway is completed, and the earlier reactants, including antigen, antibody, C1q, and $\overline{C1r}$, are not necessary for progression of the complement reaction.

Activated $\overline{C1s}$ in $\overline{C1}$ mediates the next phase of the complement reaction: formation of the key complement enzyme [Ac]$\overline{C4b2a}$ on the activator surface (Fig 10–1). [Ac]$\overline{C4b2a}$ is formed from the larger fragments of C4 and C2.

C4,C2

C4 is a β_1-globulin with a molecular weight of 206,000, and C2 is a β_1-globulin with a molecular weight of 117,000 (Table 10–2). Formation of the bimolecular complex of C2 and C4 occurs only after both of these molecules have been cleaved by $\overline{C1s}$ or $\overline{C1}$. In the case of C4, $\overline{C1}$ cleaves a single peptide bond located in the larger of the 3 polypeptide chains of this molecule, the α chain (Fig 10–3). This reaction leads to formation or generation of a labile binding site in the larger fragment of C4,C4b, which enables it to bind to activators for a brief period after generation. It has recently been shown that the α chain of C4, like C3 as described below, contains an unprecedented internal thioester bond formed between a glutamyl and a cysteinyl residue. It is probable, in analogy to C3, that $\overline{C1}$-mediated cleavage of the α chain is followed by stress-induced hydrolysis of the thioester bond. This would permit the reactive acyl group of the glutamyl residue to form a covalent bond with a reactive hydroxyl or amino group on the surface of the activator. C4a, the small peptide produced by $\overline{C1}$ cleavage of

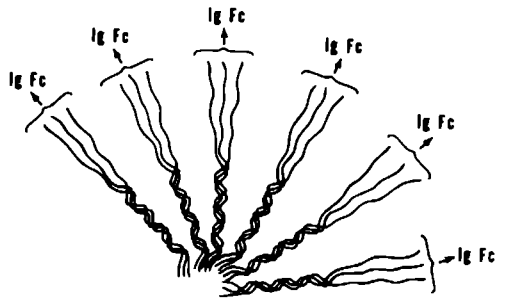

Figure 10–2. Schematic model of the C1q molecule showing the triple helical structure of a portion of each of the 6 subunits. The IgG or IgM binding sites appear to be in the noncollagenous portion of the subunits.

C4, has biologic activity. Cleavage of C2 by $\overline{C1}$ also generates a labile binding site of unknown chemical composition in the larger C2 fragment, C2a, which allows it to bind to C4b. Magnesium ions are also required for formation of the C4b2a complex. The molecular weight of $\overline{C4b2a}$ is 280,000. Formation of $\overline{C4b2a}$ is not an efficient process; the majority of the C2 and C4 molecules entering into this reaction lose their labile binding sites before achieving union with membranes or with one another and diffuse away as inactive reaction products.

$\overline{C4b2a}$ is a proteolytic enzyme that assumes the role of continuing an ongoing complement reaction, and earlier reacting components are no longer required after it has been formed (Fig 10–1). $\overline{C4b2a}$, also termed C3 convertase, cleaves and thereby activates the next reacting component of the sequence, C3. The enzymatic site resides in the C2 moiety of the complex.

C3

C3, the substrate of $\overline{C4b2a}$, is a β_1-globulin with a molecular weight of 185,000. C3 is cleaved at a single site located near the amino terminus of the larger (α) chain of the molecule (Fig 10–4). The smaller of the 2 resulting fragments, C3a, is a biologically potent peptide that will be discussed later. A labile binding site is generated in the larger fragment, C3b, which enables this molecule to attach to membranes at sites near to but distinct from those utilized by antibody and $\overline{C4b2a}$. The chemical nature of the labile binding site has been recently defined. C3 has been found to contain the amino acid sequence –Cys-Gly-Glu-Glu–with the Cys and second Glu residues joined by a thioester bond. With cleavage of C3 into C3a and C3b, the thioester apparently undergoes stress-mediated hydrolysis, and the reactive acyl group of the glutamyl residue forms a covalent bond with a reactive hydroxyl or amino group on the activator surface. A major proportion of C3 molecules undergoing cleavage fail to achieve binding. In all probability, these represent molecules in which the acyl group of the glutamyl residue has reacted with water.

The attachment of C3b to membranes in the vicinity of $\overline{C4b2a}$ molecules leads to generation of the last enzyme of the classic pathway, $\overline{C4b2a3b}$. This enzyme has C5, a β_1-globulin with a molecular weight of 191,000, as its natural substrate.

The classic complement pathway thus consists of a series of enzyme-substrate and protein-protein interactions that lead to the sequential formation of several complement enzymes. It should be emphasized that the reactions involved are highly specific, and other molecules have not been found which can substitute for the required complement components. In addition, as the reactions are enzymatically mediated, there is a considerable turnover of molecules of C2, C3, C4, and C5 at the respective steps in the reaction and an accumulation of reaction products free in plasma. Since some of these reaction products have biologic activity, it is evident that a relatively small stimulus to complement activation may lead to considerable generation of these biologically active products.

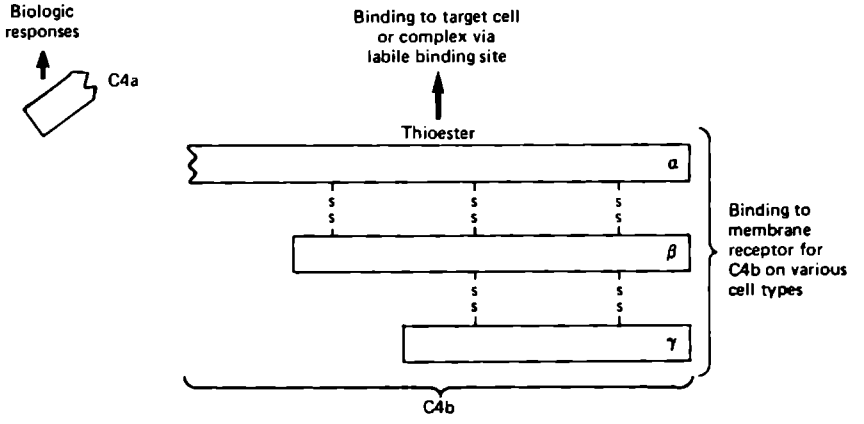

Figure 10–3. Schematic model of the C4 molecule

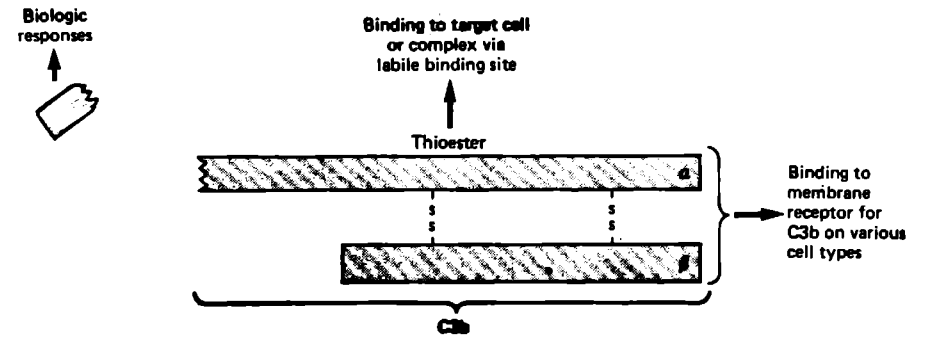

Figure 10–4. Schematic model of the C3 molecule.

THE ALTERNATIVE COMPLEMENT PATHWAY

The alternative pathway was originally described as the properdin system, a group of proteins involved in resistance to infection, which was similar to, but distinct from, complement. The properdin system was found to be involved in the destruction of certain bacteria, the neutralization of some viruses, and the lysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria. The system did not seem to require specific antibody. Several of the factors involved in the system were identified and isolated in a partially purified state. These included properdin; factor A, a high-molecular-weight protein similar in certain properties to C4 which was destroyed by treatment of serum with hydrazine; and a heat-labile substance (factor B), similar to but distinct from C2. Investigations indicate that the recently identified alternative pathway of complement activation is identical to the properdin system. Properdin was isolated and found to be a glycoprotein with a molecular weight of 220,000, having the electrophoretic mobility of a γ_2-globulin (Table 10–2). Factor A has been identified as C3. Factor B has several synonyms as a result of its involvement in systems subsequently shown to be different activities of the alternative pathway (Table 10–2); it is a β_2-globulin with a molecular weight of 93,000. The other proteins of the pathway are factor D, an α-globulin with a molecular weight of 24,000; and factors I and H, β-globulins with molecular weights of 88,000 and 150,000, respectively.

Activation of the alternative pathway proceeds in a different manner from that of the classic pathway. An initial requirement for activation is the presence of C3b, which is undoubtedly continuously generated in small amounts in the circulation. This most likely occurs following water-induced cleavage of the above-described thioester bond in C3, thus forming C3*, which reacts with factors B and D to generate a fluid phase enzyme able to cleave C3 into C3a and C3b (Fig 10–1). It is possible also that C3 in the circulation is cleaved by a loose complex of native C3 and factor B, an enzyme of the coagulation or fibrinolytic systems, or a tissue enzyme. While most of the newly generated C3b remains in the fluid phase, some binds to various cellular surfaces. In either case, this C3b is rapidly inactivated by the control proteins, factors I and H, which cleave it. This steady-state condition with low-grade continuous turnover of C3 coupled with rapid inactivation of the newly formed C3b is greatly modified by the introduction of particulate activators of the alternative pathway, such as insoluble polysaccharides and certain cells. As with other cells and tissues exposed to plasma, some of the C3b being continuously generated becomes deposited on the surface of the activator. However, C3b deposited on activators, in contrast to nonactivators, is "protected" from destruction by factors I and H. This surface-bound protected C3b interacts with factors B and D and forms an enzyme termed $\overline{C3bBb}$. This surface-bound enzyme is able to cleave very large amounts of C3. Considerable amounts of this newly generated C3b arrive on the surface of the activator, interact with additional factors B and D, and form more $\overline{C3bBb}$. There is thus at this stage a positive feedback mechanism that amplifies the initial stimulus and leads to increased C3 cleavage (Fig 10–5). Furthermore, the $\overline{C3bBb}$ enzyme molecules are rendered functionally more efficient by properdin, which binds to the complex and stabilizes it by slowing the spontaneous dissociation of factor Bb. The cyclic amplifying system, in conjunction with the crucial protected surface, represents the key events in activation of the alternative pathway.

Many of the C3b molecules generated by the surface-bound $\overline{C3bBb}$ or $\overline{C3bPBb}$ enzymes bind to the surface of the activator particle in close proximity to these enzymes. This results in the formation of modified enzymes, $\overline{C3b_nBb}$ or $\overline{C3b_nPBb}$ ($n > 1$), which are able to cleave C5 and initiate the membrane attack mechanism. The catalytic site of these enzymes resides in the factor B moiety.

The alternative pathway may also be activated by an isolated protein obtained from cobra venom. This protein appears to represent cobra C3b. This substance has been extensively used to deplete complement activity in vivo for the purpose of studying its biologic function. This protein, cobra venom factor, forms a

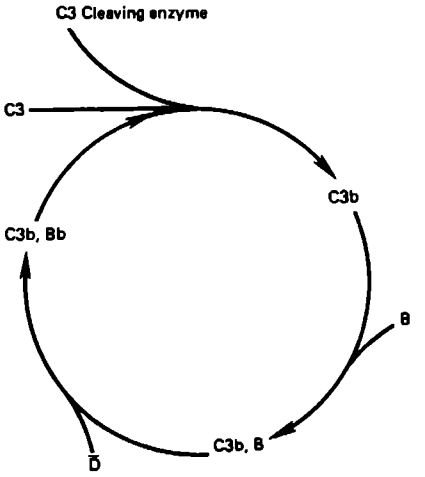

Figure 10–5. The C3b-dependent positive feedback mechanism.

firm complex with factor B in the presence of magnesium ions. Factor B, altered slightly by incorporation into the complex, is susceptible to cleavage and activation by factor D. A C3 cleaving enzyme is thus generated.

A pathologic member of this pathway, C3 nephritic factor (C3 NeF), is found in the circulation of some patients with hypocomplementemic mesangiocapillary nephritis. This protein forms a fluid phase, C3 converting enzyme, together with native C3 and factors B and D. It is an autoantibody directed to the $\overline{C3bBb}$ complex of the alternative pathway.

There are numerous analogies in physicochemical properties of the factors of the alternative and classic complement pathways and the mechanisms of activation of these proteins. $\overline{C1s}$ is similar to factor $\overline{D}$ in that both are serine esterase enzymes. $\overline{C1s}$ cleaves C4 and C2, and the larger fragment of each is incorporated into a new enzyme in the presence of magnesium. Factor D cleaves factor B, a molecule very similar in physicochemical properties to C2, in the presence of another protein, C3b, which is physicochemically similar to C4b, and thereby mediates the magnesium-dependent formation of a new proteolytic enzyme. These similar complex enzymes cleave the same single peptide bond in C3. In each instance, the newly generated C3b modulates the activity of the complex enzyme, enabling it to cleave C5.

THE REACTION OF C5–C9: THE MEMBRANE ATTACK MECHANISM

The terminal portion of the complement sequence is termed the membrane attack system, since C5b–9 must become membrane-bound in order for membrane changes or damage to occur. Following activation, this portion of the complement sequence may become attached to the surface of a cell bearing the activating enzyme of the classic or alternative pathways, or it may become bound to the membrane of a different cell or membrane not bearing any previously reacted complement components. The latter is an example of bystander lysis of cells.

The complement attack mechanism is initiated on cleavage of C5 by $\overline{C4b2a3b}$, $\overline{C3b_nBb}$, $\overline{C3b_nPBb}$, or certain enzymes such as plasmin (Fig 10–1). The activation reaction results in generation of a small biologically active peptide, C5a (MW 11,000), and a larger fragment, C5b (MW 180,000) (Fig 10–6). C5b has the ability to bind C6 and C7, thus forming a firm trimolecular complex, C5b67. This complex has a transient ability to bind to membranes. However, this process is modulated by S protein, a normal serum protein with a molecular weight of 80,000. S protein serves as a natural inhibitor by binding to the membrane binding site of the C5b67 complex. The C5b67 molecules that interact with S protein are inactivated with regard to participation in cytolysis, as the SC5b67 complex is unable to attach to membranes. Each membrane-bound C5b67 complex possesses a binding site for a molecule of C8, a γ_1-globulin with a molecular weight of 151,000. Membrane leakage begins at this stage; however, the cytolytic process is greatly accelerated by the attachment of C9, an α-globulin with a molecular weight of 71,000, to the membrane bound tetramolecular C5b678 complex. The C5b6789 complex (containing one molecule of C5b, C6, C7, C8, and several molecules of C9) represents the fully assembled cytolytic principle of the complement system.

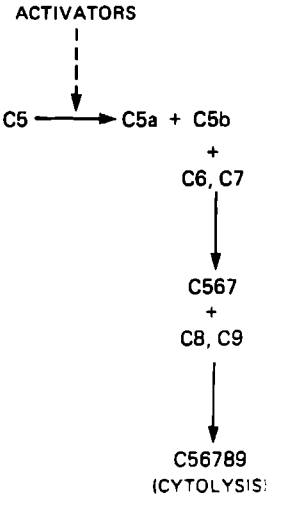

Figure 10–6. Schematic diagram of the membrane attack mechanism.

CONTROL MECHANISMS OF THE COMPLEMENT SYSTEM

Uncontrolled activation of the complement system is prevented by the lability of the activated combining sites generated at multiple stages of the complement reaction, including the reaction steps involving C2, C3, C4, and C5, and by time- and temperature-dependent dissociation of some of the active complexes such as the $\overline{C3bBb}$, $\overline{C4b2a}$, and $\overline{C4b2a3b}$ complexes. In addition, several serum proteins have been identified that serve to modulate and limit activation of the complement system. These proteins bind to or enzymatically attack only the specifically activated forms of the components.

$\overline{C1}$ inhibitor ($\overline{C1}$ esterase inhibitor) is a multispecific serum enzyme inhibitor with a molecular weight of 105,000 and the electrophoretic mobility of an α_2-globulin. This enzyme inhibitor inhibits not only $\overline{C1}$ but also the fibrinolytic enzyme plasmin, the kinin-forming system enzyme kallikrein, and the coagulation system enzymes Hageman factor (factor XII) and factor XI. $\overline{C1}$ inhibitor inhibits the enzymatic activity of $\overline{C1}$ and its $\overline{C1r}$ and $\overline{C1s}$ subunits by rapidly forming firm, essentially irreversible stoichiometric complexes. $\overline{C1}$ inhibitor does not bind to proenzyme C1 or C1s. The site of attachment is on the light chains of $\overline{C1r}$ and $\overline{C1s}$. Classic pathway activation proceeds past the $\overline{C1}$ inhibitor blockade when the stimulus to activation is so intense that $\overline{C1}$ molecules succeed in forming $\overline{C4b2a}$ sites before becoming inactivated by $\overline{C1}$ inhibitor molecules or when the available $\overline{C1}$ inhibitor has been consumed. However, activation of the kinin-forming, coagulation, or fibrinolytic systems would also be expected to facilitate activation of the complement system by consuming $\overline{C1}$ inhibitor.

Another key control protein of the complement system is factor I. This serum enzyme attacks C3b free in solution or on the surface of cells and cleaves the molecule. The C3b degradation products are unable to function in the $\overline{C4b2a3b}$ or $\overline{C3bBb}$ enzymes or to participate in the cyclic C3b-dependent feedback mechanism. Another regulator that acts at the C3b stage is factor H, a serum protein that binds to C3b and accelerates the destructive action of factor I on C3b. It also possesses the ability to bind to C3b on various intermediate complexes and, finally, exerts an inhibitory regulatory action on the alternative pathway enzyme $\overline{C3bPBb}$. In whole blood, the erythrocyte C3b receptor functions in a similar manner to factor H and facilitates the degradation of C3b by factor I. C4 binding protein binds to C4b and facilitates its destruction by factor I.

Human serum also contains an enzyme, the anaphylatoxin inactivator, an α-globulin with a molecular weight of 300,000, which destroys the biologic activities of the C3a, C4a, and C5a fragments of C3, C4, and C5, respectively. Inactivation is accomplished by cleavage of the carboxy-terminal arginine from each of these molecules.

The S protein binds to the forming C5b67 complex and thus modulates the cytolytic ability of the membrane attack complex, as described earlier. Several other inhibitors or inactivators of activated complement components have been described. These substances have not yet been analyzed in detail.

MOLECULAR GENETICS OF COMPLEMENT

Application of the techniques of molecular biology to studies of complement has yielded cDNA clones for several of the complement components, including C3, C4, C5, C9, and factor B. Of these, C4 and factor B are encoded within the major histocompatibility complex (MHC) and represent class III MHC gene products. cDNA cloning has generally been accomplished by synthesizing mixed sequence oligodeoxyribonucleotide probes corresponding to known short areas of primary amino acid sequence and employing these as hybridization probes with appropriate cDNA libraries. It has been possible, from confirmed full-length cDNA clones, to obtain the nucleotide coding sequence and deduce the amino acid sequence of the translated complement proteins. Structural features such as processing and cleavage sites, carbohydrate attachment sites, signal peptides, etc, of C3, C4, C5, C9, and factor B and the location and characteristics of the thioester site in C3 and C4 have been elucidated from such data. These studies have also shown that the class III MHC gene products are not structurally related to the class I and II MHC gene products. In addition, genomic DNA sequences and certain aspects of the exon-intron structure of C3 and C4 have been obtained. The molecular basis of genetic deficiencies and polymorphisms, mRNA expression, and numerous other aspects concerned with transcription, regulation, biosynthesis, etc, are under active investigation in many laboratories.

METHODS OF DETECTION & QUANTITATION OF COMPLEMENT COMPONENTS

Certain precautions are necessary in the handling of blood specimens for complement studies. After clotting, preferably at room temperature, the serum should be separated promptly and frozen, preferably at −70 °C, to prevent loss of complement activity. Some laboratory studies require inactivation of complement in order to avoid hemolysis. This is normally achieved by heating serum at 56 °C for 30 minutes, a procedure that destroys many but not all of the complement components.

Complement activity is generally measured by assessing the ability of serum in limiting dilution to lyse sheep red cells sensitized with rabbit antisheep antibody (hemolysin). Complement titrations of this type provide an overall measure of the integrity of the classic complement pathway and of the membrane attack

mechanism. The values are expressed as 50% hemolytic complement units per milliliter (CH_5). (See Chapter 21.)

A similar assay system is employed to measure the hemolytic activity of individual complement components in isolated form or in serum. In this type of assay system, all the components except the one in question are supplied in excess and certain stringent reaction conditions are employed which are known to be optimal for each component. Under such conditions, the number of hemolytic units may be converted on a weight basis to the absolute concentration of the active complement component. This use of hemolytic activity measurements for absolute quantitation of complement components has a firm theoretical and mathematical basis, since it has been shown that the individual reaction steps of a number of the components conform to a one-hit process. Hemolytic values obtained by such titrations are expressed as site-forming units or effective molecules.

The pattern of depletion of complement components in patients' sera or following treatment of serum with potential complement-activating agents may indicate the pathway of complement activation involved. Classic pathway activation depletes C1, C4, C2, C3, and C5 and, to a lesser extent, late-acting components as indicated in Fig 10–7. Alternative pathway activation, while depleting only small amounts of C1, C4, and C2, leads to significant consumption of C3–C9 (Fig 10–8).

Many of the complement components can be quantitated in serum or other body fluids by single radial diffusion in agar. This test is based on the fact that the extent of diffusion of an antigen into agar containing specific antibody is proportionate to the absolute antigen concentration. All of the complement components having serum levels above approximately 20 μg/mL can be measured by this technique. It should be noted

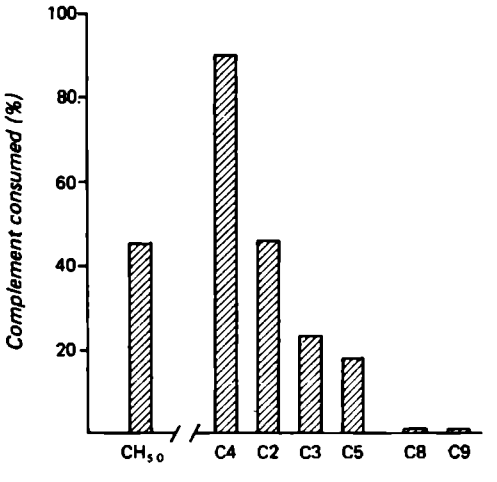

Figure 10–7. Typical component depletion pattern for classic pathway activation.

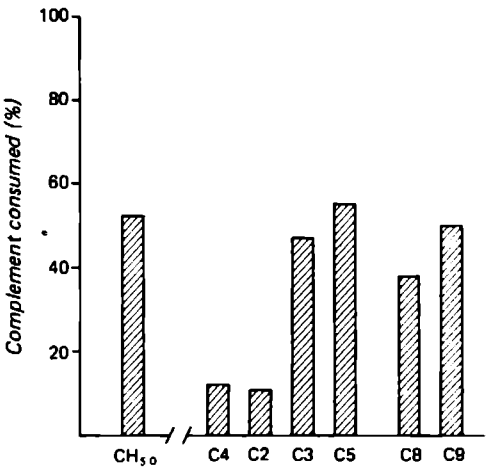

Figure 10–8. Typical component depletion pattern for alternative pathway activation.

that this method of quantitation, although very useful, does not distinguish between active and inactive complement molecules.

Assay systems have recently been developed to detect and quantitate specific features of the complement activation process. These include assays which quantitate the limited proteolytic cleavage events that characterize activation of the classic and alternative complement pathways. A second category of such tests detects and quantitates activation-specific changes in the properties of the components. A third group quantitates the protein-protein complexes that occur during complement activation. All of these tests are highly sensitive and able to detect and quantitate activation events not previously detectable. Since these various assays directly focus on activation-dependent changes in the properties of the components, they have a high degree of specificity. Finally, the tests are usable with plasma, serum, and other body fluids and thus are readily adaptable for use in the clinical laboratory. Initial studies of this type have documented the ability of these newer tests to detect complement activation in plasma from patients with various diseases. It is anticipated that wider use of these tests with clinical samples will yield new information about the role of the complement system in human diseases.

Other methods are available which are not quantitative but which furnish evidence of complement activation. One such technique involves measurement of the physicochemical status of individual complement components by the technique of immunoelectrophoresis. In the case of certain of the components such as C1s, C2, C3, C4, and factor B, the cleaved activated forms of the components are readily distinguished by an altered electrophoretic mobility. The effect of activation on the mobility of factor B is depicted in Fig 10–9. The native precursor molecule migrates as β-

Figure 10–9. Immunoelectrophoretic analysis of factor B (C3PA) in native form *(bottom)* and after activation *(top)*. Anti-factor B is in the slot, and the anode is to the right.

globulin on immunoelectrophoresis in agar, while activated factor B migrates as a γ-globulin.

Evidence of complement activation may also be obtained by showing a decrease in the immune adherence titer of the serum being investigated. Immune adherence is an agglutination reaction between a cell bearing $\overline{C4b2a3b}$ and an indicator cell, often human erythrocytes, which has a receptor for C3b. The test is generally performed by incubating dilutions of serum with antibody-coated erythrocytes during which time $\overline{C4b2a3b}$ attaches to the surface of the cells in proportion to their relative serum concentrations. Subsequently, human erythrocytes are added and the final dilution of serum giving measurable agglutination is taken as the end point.

Complement may also be demonstrated at the site of tissue damage in diseased tissues by employing specific antibody. An example is the nonimmunoglobulin Coombs test, an agglutination reaction performed with erythrocytes from patients with autoimmune hemolytic anemia and dilutions of antisera to C3, C4, or other complement components. As complement is not normally found on cells, the presence of agglutination with anticomplement sera constitutes evidence for complement activation. In a variant of this technique, complement may be localized in diseased tissues by using fluorescent or radiolabeled antisera to individual complement components. The finding of deposited complement components in the site of tissue damage constitutes evidence for activation of the complement system in the tissue.

Direct evidence of activation of the complement system in vivo may be obtained from metabolic studies with purified radiolabeled complement components. Such studies have been performed with many complement components in normal individuals and in a number of patients with diseases, including glomerulonephritis, rheumatic and autoimmune diseases, hereditary angioedema, and renal allograft rejection. These studies indicate that complement components are normally among the most rapidly metabolized of all plasma proteins, with a mean fractional catabolic rate of approximately 1.4–2% of the plasma pool per hour. Because of the very rapid turnover of complement proteins, static measurements of circulating levels of the complement components often fail to detect in vivo complement activation. Such activation and the pathway involved are, however, readily detected and quantitated by metabolic studies. Hypercatabolism has been found in patients with proliferative glomerulonephritis, systemic lupus erythematosus, seropositive rheumatoid arthritis, hereditary angioedema, and renal allograft rejection. Metabolic studies also reveal reduced synthesis of complement—C3 in some patients with membranoproliferative glomerulonephritis and C4 in some patients with IgG-IgM cryoglobulinemia and Sjögren's syndrome.

BIOLOGIC CONSEQUENCES OF COMPLEMENT ACTIVATION

Cytolytic & Cytotoxic Damage

Complement has been shown to be capable of mediating the lytic destruction of many kinds of cells including erythrocytes, platelets, bacteria, viruses possessing a lipoprotein envelope, and lymphocytes, although with greatly varying degrees of efficiency in each instance. Either complement pathway may produce cytolytic damage. Some species of complement are more efficient in producing lysis of certain cell-antibody combinations. Some cells are quite resistant to destruction by complement even in the presence of marked complement activation on the cell surface. There are many reasons why complement may fail to lyse cells, including the presence of antigenic modulation, a phenomenon whereby antibody alters the distribution of antigen on the cell surface, or a spatial arrangement of antigenic sites that does not facilitate complement activation in a region of the membrane susceptible to lysis. Lack of binding sites for the late-reacting complement components is another possible cause of failure of complement to lyse a cell. Most commonly, however, complement fails to produce lysis because of the nature and structure of the cell wall or membrane or because the cell repairs the complement-mediated damage. Factors in addition to complement may also be required, as in the lysis of gram-negative bacteria.

As the complement components free in serum become attached to the surfaces of cells and other biologic membranes, changes in membrane ultrastructure

occur (Table 10–3). There are alterations in membrane electrical charge and membrane environment due to the accumulation of complement proteins on the cell surface. Membrane swelling also occurs. Complement action produces circular lesions having a diameter of 8–12 nm in many types of membranes (Fig 10–10). These lesions, which are tubular C5b–9 complexes inserted into the cellular membrane, are the sites of lytic membrane damage. The mechanism by which the C5b–9 complex disrupts cellular lipid bilayer membranes leading to lysis involves 2 processes. The first—and probably physiologically the more important—involves reorientation of membrane lipids and phospholipids surrounding C5b–9 complexes, leading to leaky patches. The other process occurs at high multiplicities of C9 molecules within the C5b–9 complex. Under such conditions, the C5b–9 complex is tubular and contains a central channel through which ions can diffuse and mediate lysis in colloid-osmotic systems.

Figure 10–10. Electron micrograph demonstrating the circular lesions produced in the membrane of an enveloped virus by the cytolytic action of complement.

Interactions With Complement Receptors

New sites are generated or uncovered in the b fragments of C3 and C4 as a consequence of proteolytic cleavage of the molecules during complement activation (Figs 10–3 and 10–4). The labile binding sites which were uncovered or generated by proteolytic cleavage and which permit binding to membranes have been considered earlier. In addition, however, other stable reactive sites are generated in C3b and C4b and in further breakdown products of these molecules, which are recognized by various cells having specific receptors for these fragments. The secondary or responding cells bearing such complement receptors bind to these sites in the larger C3 and C4 fragments. In the instances where the C3 and C4 fragments are attached by their labile binding sites to an immune complex or other particulate complement activator, the C3 and C4 molecules directly mediate the attachment of the activator to the responding cell. This process may trigger one or more responses by the cell.

There are one or more distinct complement receptors on the surface of most of the types of cells found in blood; certain tissue cells also express complement receptors (Table 10–4). These various receptors interact only with the activated forms of specific complement components and have little (if any) ability to bind the native circulating forms of these factors. Thus, complement activation is a prerequisite for complement-receptor interactions.

Table 10–3. Consequences of attachment of complement proteins to membranes.

Accumulation of bulk of complement proteins
Changes in membrane environment and charge
Modification of membrane properties and functions
Stimulation of cellular functions
Membrane lesions and swelling
Membrane damage or disruption

There are 6 distinct receptors for C3 activation fragments located on many different types of cells. Each of these preferentially reacts with a single C3 fragment. The C3a receptor, which binds the small C3a activation peptide, is found on mast cells, PMNs, and macrophages. Engagement of the receptor triggers a number of responses by the cells, as noted in the next section.

The C3 receptor termed CR1 preferentially binds the initial C3 activation product, C3b. CR1 is found on human red blood cells, B lymphocytes, some T lymphocytes, PMNs, monocytes, macrophages, glomerular podocytes, and dendritic reticular cells in lymphoid germinal centers. CR1 is a single glycosylated polypeptide chain that exists in allotypic forms with molecular weights ranging from 160,000 to 250,000. The consequences of engagement of CR1 by activator-bound C3b differ depending on the cell type involved. Erythrocyte CR1 regulates complement ac-

Table 10–4. Specificity and cellular distribution of complement receptors.

Receptor	Primary Specificity	Cell Type
C3a	C3a, C4a	PMNs, macrophages, mast cells
CR1	C3b	Erythrocytes, PMNs, B lymphocytes, subsets of T lymphocytes, monocytes, macrophages, glomerular podocytes, dendritic reticular cells
CR2	C3d,g and C3d	B lymphocytes
CR3	C3bi	PMNs, monocytes, macrophages
CR4	C3d,g	PMNs, monocytes
C3e	C3e	PMNs
C5a	C5a	PMNs, monocytes, macrophages, mast cells
Clq	Clq	PMNs, B lymphocytes, monocytes, macrophages
H	H	PMNs, B lymphocytes, monocytes

PMN = polymorphonuclear neutrophil.

tivation and aids in the clearance of immune complexes. On certain lymphoid cells and phagocytic cells, CR1 synergizes with antibody to augment destruction of C3b-bearing complement activators. The functions of kidney and dendritic reticular cell CR1 receptors are unknown.

The C3 receptor known as CR2 binds the terminal C3 cleavage products C3d,g and C3d. This receptor is confined to B lymphocytes. The molecule is a single-chain glycoprotein with a molecular weight of 145,000. In addition to its role in binding C3d,g and C3d, CR2 has been shown to be the structure used by Epstein-Barr virus, a human herpesvirus, to attach to and infect human B lymphocytes. The physiologic functions of CR2 have not been fully elucidated.

The CR3 complement receptor binds the intermediate C3 cleavage product, C3bi. CR3, which is confined to certain cytolytic lymphocytes and phagocytic cells, is composed of 2 noncovalently linked glycosylated polypeptide chains with molecular weights of 180,000 and 90,000. CR3 is a member of a family of cell surface glycoproteins that share the same smaller polypeptide chain; the other members of the family do not interact with complement activation fragments. Interactions of complement activator-associated C3bi with CR3 on phagocytic cells enhance antibody-dependent responses, leading to destruction of the activator. Individuals genetically lacking CR3 are predisposed to recurrent life-threatening bacterial infections.

CR4 is a newly described C3 receptor that binds C3bi and C3d,g and, under certain circumstances, C3d. It is present on PMNs and monocytes. Its function is unclear.

The C3e receptor selectively reacts with a small degradation product of C3 termed C3e. It is confined to PMNs. Engagement of this receptor in vivo releases leukocytes from the bone marrow.

Both primary cleavage fragments of C4—C4a and C4b—bind to the surface of a number of types of cells. Studies indicate that C4a and C4b bind to the C3a and CR1 receptors, respectively, located on various cells.

In addition to the above-described C3 and C4 fragment receptors, there is a receptor for C5a, the primary C5 activation fragment. This receptor, one of the most important complement receptors, is found on the surface of mast cells, PMNs, monocytes, and macrophages and triggers the responses noted in the next section.

A receptor for the collagenous portion of C1q is found on PMNs, monocytes, and macrophages. The receptor is a glycoprotein-proteoglycan complex. Finally, a receptor for the control protein, factor H, is found on B lymphocytes, monocytes, and PMNs. The biologic significance of these 2 receptors is not clear.

Biologic Actions of Complement Cleavage Products

The low-molecular-weight fragments of C3, C4, and C5—C3a, C4a, and C5a, respectively—are known as **anaphylatoxins** (Table 10–5). These hormonelike peptides bind to specific receptors, as noted above, and induce smooth muscle contraction, enhance vascular permeability, release vasoactive amines such as histamine from mast cells and basophils, and induce lysosomal enzyme release from granulocytes. In addition, the C5a molecule is chemotactic; ie, it is able to induce the migration of leukocytes into an area of complement activation. The C5a molecule also has numerous other properties, which include granulocyte aggregation and activation of intracellular processes in certain cells, leading to various effects such as release of oxygen metabolites and SRS-A.

Table 10–5. Biologic effects of complement activation peptides.

C3a, C4a, C5a	Cellular release of vasoactive amines. Enhanced vascular permeability. Contraction of smooth muscle. Induced release of lysosomal enzymes. Stimulation of arachidonic acid metabolism.
C5a	Chemotaxis. Granulocyte aggregation. Stimulation of oxidative metabolism. Stimulation of SRS-A release.

Many if not all of the C3a and C4a effects appear to be due to histamine released as a consequence of C3a and C4a interaction with mast cells and basophils. These effects are abrogated by antihistamines and by the action of anaphylatoxin inactivator, or carboxypeptidase N, a serum enzyme that removes the C-terminal arginine residue from these peptides. Although antihistamines and this control enzyme inhibit some of the effects of C5a, C5a des Arg, which lacks the C-terminal arginine, retains significant chemotactic, granulocyte-aggregating, and intracellular activating ability.

C3a, C4a, and C5a have molecular weights of 9038, 8740, and 11,200, respectively. Analyses of the primary structures of these molecules indicate that they are genetically related. They also share a number of the same biologic actions as noted above. Despite these facts, the C3a and C5a anaphylatoxins interact with distinct cell surface receptors and thus are biologically distinct.

There are also other biologic consequences of activation of the complement system mediated by other complement cleavage products. These include the generation of a kinin, possibly a fragment of C2, which increases vascular permeability and contracts smooth muscle. This kinin does not function through release of histamine. It is thought to be involved in the symptomatology of hereditary angioedema. Functional C1 inactivator is genetically lacking in this disease, which is characterized by uncontrolled activation of the complement system.

BIOLOGIC SIGNIFICANCE OF THE COMPLEMENT SYSTEM

The biologic reactions considered above are individual aspects of an integrated system that is able to produce inflammation and facilitate the localization of an infective agent (Fig 10–11). Thus, the kinin and anaphylatoxin activities lead to contraction of smooth muscle, increased vascular permeability, and edema. The chemotactic agents trigger an influx of leukocytes that remain fixed in the area of complement activation through attachment to specific sites on bound C3b and C4b molecules. Phagocytosis or release of lysosomal and other constituents facilitates the destruction of an infective agent. As is evident from Fig 10–11, there are multiple backup systems which produce similar biologic activities. A minor stimulus to activation of the system produces relatively little of these biologic mediators, while a greater stimulus to activation can be visualized as leading to the generation of additional cleavage products and reactive sites on the components.

Evidence for the biologic importance of this system in host defenses has come from studies of several experimentally induced diseases in animals, from human immunologic disease processes, and from the markedly increased susceptibility to infection that characterizes some congenital or acquired deficiencies of complement components or complement regulators in humans. These disease entities bear certain hallmarks which imply the participation of complement. These include a depression in circulating levels of the complement components, the finding of complement components deposited in the site of tissue damage, and infiltration of PMNs. In animals, it has been possible to further define the pathogenic role of complement in certain conditions. One of the most telling examples is the experimental disease nephrotoxic nephritis, which is induced by the injection into an animal of antibody directed against glomerular basement membrane. The injected antibody rapidly fixes to the glomerular basement membrane, and the result is immediate structural and functional injury. Antibody attachment is rapidly followed by activation of complement, which is reflected in a fall in circulating levels and by fixation of complement components to the glomerular basement membrane, where they may be visualized by fluorescence techniques. An influx of PMNs rapidly ensues, followed by destruction of the glomerular basement membrane and proteinuria, which are consequences of the release of degradative enzymes from the leukocytes. The essential role of complement in leading to the influx of leukocytes and facilitating their localization is shown by the fact that infiltration and tissue damage are prevented if the antibody is first rendered unable to fix complement or if the animal is

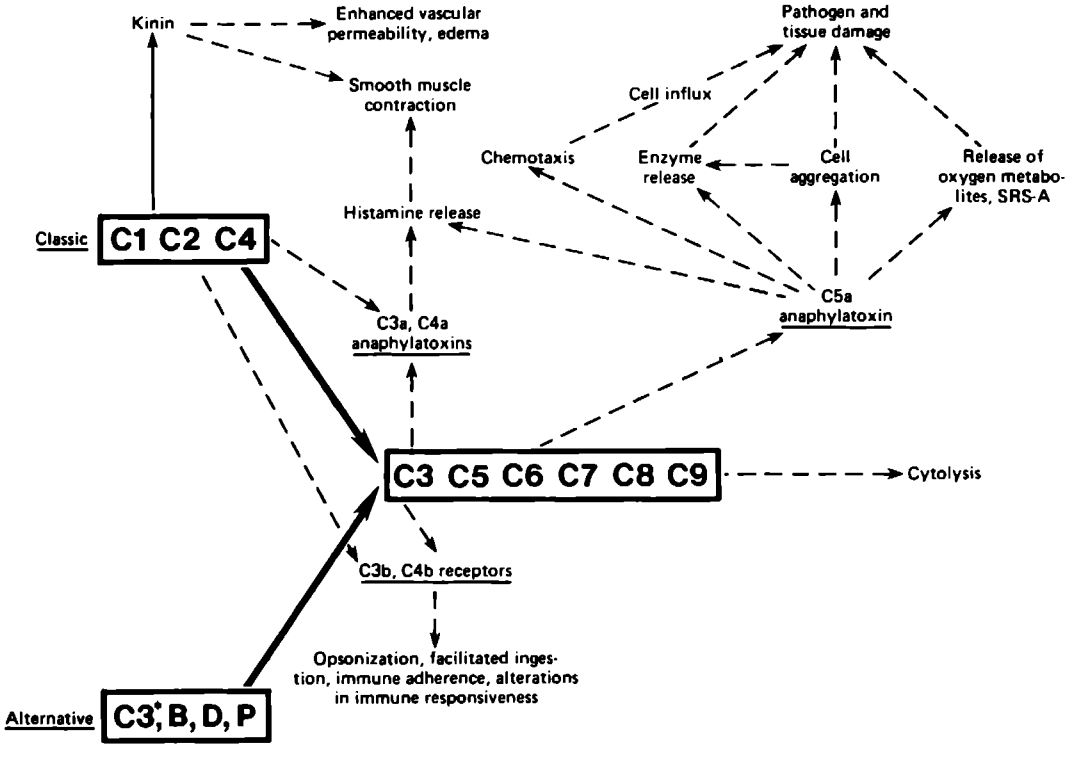

Figure 10–11. Biologic consequences of complement activation. (P = properdin; B = factor B; D = factor D.)

Table 10–6. Human disorders associated with congenital complement deficiencies.

C1q	Systemic lupus erythematosus or similar syndrome, hypogammaglobulinemia, nephritis
C1r	Renal disease, systemic lupus erythematosus or similar syndrome, recurrent infections, rheumatoid disease
C1s	Systemic lupus erythematosus
C4	Systemic lupus erythematosus
C2	Arthralgia, systemic lupus erythematosus or similar syndrome, nephritis, susceptibility to infection
C3	Recurrent infections with pyogenic bacteria
C5	Systemic lupus erythematosus, recurrent infections, recurrent gonococcal infections
C6	Recurrent gonococcal and meningococcal infections, Raynaud's phenomenon
C7	Recurrent gonococcal and meningococcal infections, glomerulonephritis, Raynaud's phenomenon
C8	Recurrent gonococcal and meningococcal infections, systemic lupus erythematosus
$C\bar{1}$ In	Hereditary angioedema
C3b In	Repeated infections, recurrent infections with pyogenic bacteria

depleted in vivo of C3. Similar mechanisms are involved in the inflammatory component of a number of human diseases, including several types of glomerulonephritis, rheumatoid arthritis, autoimmune hemolytic anemias, and others. Metabolic studies with purified radiolabeled complement components have documented and quantitated in vivo complement activation in these and other diseases as noted earlier.

The physiologic role of complement in the maintenance of a normal state of health is dramatically illustrated by the predisposition to disease or susceptibility to infection that characterizes congenital deficiency of certain of the complement components or their regulators (Table 10–6). Congenital deficiency of C1 inactivator leads to uncontrolled activation of the classic pathway and hereditary angioedema. Over half of the more than 50 reported individuals with hereditary deficiencies of the classic pathway—C1r, C1s, C4, and C2—are clinically ill and suffer from several diseases, including systemic lupus erythematosus, glomerulonephritis, and repeated infections. Most of the relatively few individuals found with genetic deficiencies of C3 or its regulator, C3b inactivator, suffer from recurrent life-threatening infections. About half of the 20 individuals with inherited deficiencies of the terminal components—C5, C6, C7, and C8—have recurrent infections with *Neisseria* organisms (gonococcus or meningococcus).

The genes for C2, C4, and factor B (but not other complement components) are coded within the major histocompatibility complex (MHC, HLA) in humans (see Chapter 6), but their relationships to other MHC gene products are not known. It is not entirely clear how the absence of a complement component or a regulator predisposes to disease. Most of the genes within this region are of fundamental importance to immune recognition, regulation, and responses (see Chapter 6), and these unexplained relationships suggest major but as yet unknown physiologic roles for the complement system in vivo.

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11 Autoimmunity

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Recent major advances in cellular immunology, molecular biology, and genetics have strongly influenced current thinking about autoimmunity. These advances have increased our understanding of the basic aspects of antibody diversity, the generation of cellular and humoral immune responses and their interdependence, the mechanisms of tolerance induction, and the means by which reactivity develops against autoantigenic constituents.

Since 1900, the central dogma of immunology has been that the immune system does not normally react to self. This phenomenon, described originally by Ehrlich, is accepted today as immunologic tolerance to self components, an obvious necessity for health. Accordingly, autoimmunity defines a state in which the natural unresponsiveness or tolerance to self terminates. As a result, antibodies or cells react with self constituents, thereby causing disease. The above definition implies that responses against self do not occur normally and that if they do occur with sufficient magnitude and duration, the outcome is harmful to the host. However, it has recently become apparent that autoimmune responses are not as rare as once thought and that not all autoimmune responses are harmful. In fact, current argument emphasizes that certain forms of autoimmune responses such as recognition of cell surface antigens encoded by the major histocompatibility complex (MHC) and of anti-idiotypic responses against self idiotypes, unlike the "horror autotoxicus" responses of Ehrlich, are important, indeed essential, for the diversification and normal functioning of the intact immune system. Therefore, a distinction between "horror autotoxicus" and normal or positive autoimmune responses is in order.

It is now recognized that an abnormal autoimmune response is sometimes a primary cause and at other times a secondary contributor to many human and animal diseases. Clinically, the wide spectrum of autoimmune diseases has been divided into systemic, or "non-organ-specific diseases" and organ-specific ones (Table 11–1). Types of autoimmune diseases frequently overlap, and more than one autoimmune disorder tends to occur in the same individual, especially in persons with autoimmune endocrinopathies. For unknown reasons, autoimmune syndromes may also be associated with lymphoid hyperplasia, malignant lymphocytic or plasma cell proliferation, and immunodeficiency disorders such as hypogammaglobulinemia, selective IgA deficiency, and complement component deficiencies. Moreover, autoantibodies sometimes develop as part of the aging process. Non-organ-specific autoimmune diseases, epitomized by systemic lupus erythematosus (SLE), are characterized by autoimmune responses directed against widely distributed self-antigenic determinants. Although a given non-organ-specific disease usually involves many self antigens, such diseases may also develop following abnormal immune responses against only one antigenic target that is expressed in different organs. One example of such an antigen is determinants on basement membranes at diverse sites. In contrast to generalized autoimmune diseases, organ-specific diseases (eg, certain forms of thyroiditis) result from abnormal responses directed against an antigen that is confined to a given organ. It is not known what determines the extent of autoimmune responses, the number of autoantigens that elicit them, or the target organ. In many instances, it is not clear whether autoimmune responses are directed against unmodified self antigens or self antigens that have been modified by any of numerous agents such as viruses and haptenic groups.

There is as yet no established unifying concept to explain the origin and pathogenesis of the various autoimmune disorders. Studies in experimental animals support the notion that autoimmune diseases may result from a wide spectrum of genetic and immunologic abnormalities which differ from one individual to another and may express themselves early or later in life depending on the presence or absence, respectively, of many superimposed exogenous (viruses, bacteria) or endogenous (hormones, abnormal genes) accelerating factors.

IMMUNOPATHOLOGIC MECHANISMS IN AUTOIMMUNE DISEASES

Three main immunopathologic mechanisms act to mediate autoimmune diseases, though in any given disorder more than one may sometimes be in operation:

(1) The first mechanism is the action of autoantibody on unmodified or modified structures on cell sur-

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Table 11–1. Autoimmune diseases.

	Autoantibody	Method of Detection
Organ-specific diseases		
Myasthenia gravis	Anti-acetylcholine receptor.	Immunoprecipitation of ^{125}I-α-bungarotoxin conjugated acetylcholine receptors.
Graves' disease (diffuse toxic goiter)	Thyroid-stimulating immunoglobulin (TSI) or anti-TSH receptor autoantibody.	Bioassay; measurement of adenylate cyclase activity after incubation of thyroid tissue with immunoglobulin from patient's serum, radioreceptor assay for antibodies competing with TSH for the receptor on thyroid membranes.
Hashimoto's thyroiditis	Antibodies to thyroglobulin and to microsomal antigens.	Radioimmunoassay, tanned erythrocyte agglutination, complement fixation, immunofluorescence assay.
Insulin-resistant diabetes associated with acanthosis nigricans	Anti-insulin receptor.	Inhibition of ^{125}I-insulin binding to receptors on monocytes or adipocytes; activation of lipogenesis in adipocytes.
Insulin-resistant diabetes associated with ataxia-telangiectasia	Anti-insulin receptor.	
Allergic rhinitis, asthma, functional autonomic abnormalities	Antibodies to β_2-adrenergic receptors.	Binding of ^{125}I-protein A to lung membranes preincubated with sera; ability of plasma to inhibit binding of ^{125}I-iodohydroxybenzylpindolol (HYP) to calf lung membranes; immunoprecipitation of soluble receptors complexed with ^{125}I-HYP in the presence of propranolol.
Juvenile insulin-dependent diabetes	Antibodies to islet cells; anti-insulin antibodies.	Immunofluorescence assay; competitive inhibition of insulin binding, stimulation of adipocytes.
Pernicious anemia	Antibody to gastric parietal cells and to vitamin B_{12}-binding site of intrinsic factor.	Immunofluorescence assay; radioimmunoassay.
Addison's disease	Antibodies to adrenal cells.	Immunofluorescence assay.
Idiopathic hypoparathyroidism	Antibodies to antigens of parathyroid cells.	Immunofluorescence assay.
Spontaneous infertility	Antibodies to sperm.	Agglutination and immobilization of spermatozoa.
Premature ovarian failure	Antibodies to interstitial cells and corpus luteum cells.	Immunofluorescence assay.
Pemphigus	Antibodies to intercellular substance of skin and mucosa/desmosome.	Immunofluorescence assay.
Bullous pemphigoid	Antibodies against basement membrane zone of skin and mucosa.	Immunofluorescence assay.
Primary biliary cirrhosis	Antibodies to mitochondrial antigens.	Immunofluorescence assay.
Autoimmune hemolytic anemia	Anti-red blood cell antibodies.	Direct and indirect Coombs tests.
Idiopathic thrombocytopenia purpura	Antiplatelet antibodies.	Immunofluorescence assay.
Idiopathic neutropenia	Antineutrophil antibodies.	Agglutination, immunofluorescence assay.
Vitiligo	Anti-melanocyte antibodies.	Immunoprecipitation, immunofluorescence assay.
Osteosclerosis and Meniere's disease	Anti-collagen type II antibodies.	Radioimmunoassay.
Chronic active hepatitis	Antinuclear antibodies; anti-hepatocyte antibodies.	Immunofluorescence assay.
Ulcerative colitis	Anti-colon antibody.	Immunofluorescence, Western blot electrophoresis.
Systemic diseases ("non-organ-specific")		
Goodpasture's syndrome	Anti-basement membrane antibodies.	Immunofluorescence assay, radioimmunoassay.
Rheumatoid arthritis	Anti-γ-globulin antibodies. Antibodies to EBV-related antigens (RANA).	Sensitized-SRBC agglutination, latex-immunoglobulin agglutination, radioimmunoassay, immunofluorescence assay, immunodiffusion.

Table 11–1 (cont'd). Autoimmune diseases.

	Autoantibody	Method of Detection
Systemic diseases ("non-organ-specific") (cont'd)		
Systemic lupus erythematosus	Antinuclear antibodies.	Immunofluorescence assay.
	Anti-dsDNA, anti-ssDNA, anti-Z-DNA.	Farr assay, solid-phase enzyme and radioimmunoassay, hemagglutination, counterelectrophoresis.
	Anti-Sm antibodies (U1, U2, U4, U5, U6 RNP).	Hemagglutination, immunodiffusion, radioimmunoassay, gel electrophoresis.
	Anti-SS-A/Ro (50%), anti-SS-B/La (15%).	Radioimmunoassay, immunodiffusion, gel electrophoresis.
	Antihistone antibodies.	Radioimmunoassay, immunofluorescence.
	Antilymphocyte antibodies.	Immunofluorescence assay, cytotoxicity.
	Anti-red blood cell antibodies.	Coombs test.
	Antiplatelet antibodies.	Immunofluorescence assay.
	Anti-neuronal cell antibodies.	Immunofluorescence assay.
Sjögren's syndrome	Anti-SS-A/Ro antibodies, anti-SS-B/La antibodies.	Radioimmunoassay, immunodiffusion, gel electrophoresis.
Mixed connective tissue disease (MCTD)	Anti-RNP antibodies (U1-RNP).	Immunodiffusion, gel electrophoresis, hemagglutination.
Scleroderma/CREST	Anti-Scl-70 (topoisomerase I)/anti-centromere (CENP-B) antibodies.	Immunodiffusion, ELISA, gel electrophoresis/immunofluorescence.
Polymyositis	Anti-PM-1 antibodies, anti-Jo-1 (histidyl-tRNA synthetase).	Immunodiffusion, gel electrophoresis.

faces. Destruction of cells or tissues ensues, usually because of the presence of complement but also sometimes as a result of antibody-mediated cellular cytotoxicity. In some instances, autoantibodies directed against functional cellular receptors stimulate or inhibit specialized cellular functions without associated cell destruction.

(2) Second, autoantigen-autoantibody immune complexes may form in intercellular fluids or in the general circulation and ultimately mediate tissue damage. Such complexes, depending on their size—which is determined primarily by the ratio of the 2 reactants—may circulate widely and be deposited in tissues throughout the body, especially those with large filtering membranes (kidney, joint, choroid plexus). Complement factors as well as granulocytic and monocytic cells are then attracted to the sites of immune complex deposition, and their involvement leads to cell death.

(3) Third, the disease process may be caused by sensitized T lymphocytes. These lymphocytes produce tissue lesions by poorly understood mechanisms which presumably involve the release of destructive lymphokines or which attract other destructive inflammatory cell types to the lesion.

Examples of the first type of autoimmune diseases are autoimmune hemolytic anemias, neutropenias, lymphopenias, and thrombocytopenias as well as antibasement membrane antibody-caused diseases, a variety of autoimmune endocrinopathies, and anti-receptor-mediated diseases (Table 11–1). The immunopathologic mechanisms of some of these diseases will be summarized below. Hemolytic anemias can be idiopathic or secondary to such factors as viral infections and drugs, and the cause may be warm- or cold-reactive autoantibodies that are detectable bound to red cell surfaces or in serum samples examined by direct and indirect hemagglutination assays. Lymphopenias, neutropenias, and thrombocytopenias are frequent secondary manifestations of autoimmune disorders such as SLE and rheumatism in which antilymphocyte, anti-polymorphonuclear cell, and antiplatelet antibodies often develop. Goodpasture's syndrome, characterized by glomerulonephritis and pulmonary hemorrhage, is caused by anti-basement membrane autoantibodies; these antibodies can be found deposited uniformly along the membrane, resulting in a smooth continuous linear pattern (see Chapters 26 and 28). A variety of endocrinopathies may result from autoantibodies directed against antigens on endocrine glands, hormones produced by them, or receptor sites for the hormones. For example, Addison's disease may be the result of antiadrenal autoantibodies. Most patients with juvenile-onset (insulin-dependent) diabetes have islet cell antibodies in their sera (64,000-MW protein on the surface of the beta cell), and patients with Hashimoto's thyroiditis and primary myxedema have antibodies to thyroglobulin, microsomal protein, and other thyroid constituents. It is of interest that over 30% of patients with autoimmune thyroid disease have concomitant gastric parietal cell antibodies in their sera, whereas thyroid antibodies have been demonstrated in up to 50% of pernicious anemia patients. Parietal cells in many ways behave like endocrine cells, since they secrete intrinsic factor in response to stimulation by gastrin. Absence of intrinsic factor leads to malabsorption of vitamin B_{12}. Pernicious anemia may develop as a result not only of autoantibodies against parietal cells but also of autoantibodies specific for intrinsic factor.

A most interesting group of autoimmune endocrinopathies and other autoimmune diseases is caused by autoantibodies against functional cell surface receptors. Antireceptor antibodies are known to

underlie the pathogenesis of at least 4 diseases: (1) myasthenia gravis, with antibodies produced against the acetylcholine receptors of neuromuscular junctions; (2) Graves' disease, in which antibodies against the thyroid receptors for thyroid-stimulating hormone (TSH) develop; (3) the syndrome of acanthosis nigricans, in which profound insulin resistance results from the production of anti-insulin receptor antibodies; and (4) ataxia-telangiectasia, another autoimmune disorder in which one finds antibodies against the insulin receptors. In each case, the antibody (usually IgG, but sometimes other immunoglobulins) competes with neurotransmitter or hormone for binding sites on the cell surface. Attachment of antibody to the receptor can result in a variety of biologic effects such as (1) blocking of function by hastening degradation of the receptor, as in myasthenia gravis; (2) mimicking the action of a normally activated receptor, as in Graves' disease and certain cases of acanthosis nigricans with diabetes; and (3) blocking hormone binding, thereby inducing resistance to the hormone, as in ataxia-telangiectasia.

Myasthenia gravis is a neuromuscular disorder manifested by weakness, fatigue of voluntary muscles, and often remarkable patient responsiveness to anticholinesterase drugs. The functional defect in neuromuscular transmission observed in this disease is localized in the postsynaptic surface of the neuromuscular junction. The structure of the neuromuscular junction is altered, and the number of functional acetylcholine receptors decreases—all brought about by anti-acetylcholine receptor antibodies. These autoantibodies interact with acetylcholine receptors located on the postsynaptic membrane at or near the acetylcholine binding site, and this interaction leads to blockade, greatly increased receptor interiorization, and subsequent degradation as well as focal lysis in the presence of complement. The disease can be transmitted to experimental animals by serum IgG of myasthenic patients.

Graves' disease, characterized by overproduction of thyroxine and triiodothyronine, is probably caused by thyroid-stimulating immunoglobulins (TSI), which stimulate the thyroid gland through a reaction with the cell receptor for TSH. This reaction activates adenylate cyclase inside the cell membrane, initiating increased activity by protein kinases, which leads to increased secretion of thyroid hormones.

In a few nonobese patients with type B acanthosis nigricans and diabetes, insulin is often present at normal or above-normal levels, but its binding to specific receptors is greatly diminished. The insulin receptors, although present in normal numbers, are almost completely inactivated by autoantibody directed against them. The antibodies attach themselves to the receptors, probably at some site adjacent to the receptor rather than at the actual insulin-binding site, changing the receptor's total structure in such a way that it can no longer bind insulin tightly. Such autoantibodies may not only block and desensitize the receptors, but when bound they can also mimic insulin's action on target cells. Although this insulin-mimicking effect lasts only a short while, it indicates that the information for turning on cells is in the receptor rather than in insulin itself. Insulin-resistant diabetes is found in approximately 60% of patients with ataxia-telangiectasia; this has been attributed to the presence in serum of blocking anti-insulin receptor antibodies. Recent studies indicate that over 50% of patients with juvenile-onset insulin-dependent diabetes mellitus (IDDM) also express anti-insulin receptor antibodies in addition to the anti-islet cell autoantibodies. However, unlike the antireceptor antibodies in acanthosis nigricans patients, those in IDDM patients are predominantly IgM rather than IgG.

Recent findings suggest that antireceptor autoantibodies may be responsible for many other syndromes. For example, autoantibodies to β_2-adrenergic receptors have been identified occasionally in sera of patients with bronchial asthma or allergic rhinitis. Receptor blockade by β_2-receptor antibodies could upset the balance between β-receptor-induced relaxation of airway smooth muscle and the opposing influence of other mediators such as α-receptor agonists, histamine, prostaglandins, and acetylcholine. The β-receptor antibodies might also reduce receptor density on smooth muscle cells by hastening receptor degradation, as with acetylcholine receptor in myasthenia gravis. A recent suggestion is that such autoantibodies may be more prevalent than originally thought and that they may play an important role in the pathogenesis of inherent functional autonomic abnormalities.

Among the non-organ-specific autoimmune diseases, the most prominent is systemic lupus erythematosus (SLE). This prototypical autoimmune disease is characterized by a variety of autoimmune responses and manifestations of immune complex disease such as glomerulonephritis, vasculitis, and nonerosive polyarthritis. Among the various autoantibodies encountered, the most notable are those against nuclear components such as DNA, deoxyribonucleohistone, histone, RNA, nucleolar antigens, and components of the soluble nuclear extracts, among which the most prominent are ribonucleoprotein antigen and Sm antigen. High titers of antibody to double-stranded DNA and to Sm are found mainly in patients with SLE and can be considered diagnostic. Some antinuclear antibodies, especially antibodies to single-stranded DNA, occur with various frequencies and titers in other rheumatic diseases as well. In SLE, there also may be anti-red cell and antiplatelet antibodies that cause hemolytic anemia and thrombocytopenia, anti-intermediate filament (vimentin) autoantibodies, as well as antilymphocyte antibodies directed against T and B cells and anti-neuronal cell antibodies that may play some role in the central nervous system manifestations of SLE. Recent studies of monoclonal anti-DNA antibodies derived from humans and mice with SLE have suggested to some investigators that the autoantibody spectrum of lupus may not be as broad as once thought, since some monoclonal antibodies react with numerous substances (cardiolipin, phosphatidic acid,

phosphatidylglycerol, lupus anticoagulant factor, polynucleotides) whose backbones contain diester-linked phosphate groups. It was suggested that some of the diverse serologic abnormalities in patients with SLE may result from the binding of certain autoantibodies to a phosphodiester-containing epitope present in diverse biologic molecules distributed widely throughout the body. Recently, similar possibilities have been suggested for the pathogenesis of autoimmune polyendocrinopathies. Certain monoclonal antibodies derived from such patients exhibited multiple organ specificity and suggested either reactivity with the same molecule present in several organs or with common antigenic determinants on different molecules in multiple organs.

Recent studies have implied that antinuclear antibodies in sera of humans and mice with SLE may have a much more fundamental effect than just complexing with antigen in serum and depositing onto tissues. Thus, experiments in SLE-prone strains of mice and humans with SLE have disclosed the presence in sera of autoantibodies directed not only against the classic right-handed helical DNA (B-DNA) but also against left-handed helical DNA (so-called Z-DNA). Z-DNA was proved to be strongly immunogenic in experimental animals—unlike B-DNA, for which such animals exhibit strong tolerance. Theoretically, Z-DNA is an inactive methylated form of DNA that, upon demethylation and activation, becomes involved in gene regulation. Moreover, in recent research, autoantibodies to Sm and RNP interacted with a type of small RNA complexed with protein. This small RNA was highly conserved among the species tested. The anti-RNP antibodies recognized small nuclear ribonucleoprotein particles (snRNP) that contained U1 RNA, whereas the anti-Sm antibodies recognized, in addition to U1 RNA, particles containing RNA species U2, U4, U5, and U6. Autoantibodies reactive with RNP bind to the protein rather than the RNA component. Experiments with an in vitro system containing HeLa cells infected with adenovirus revealed that both anti-RNP and anti-Sm antibodies could inhibit the appearance of spliced mRNAs by interfering with the function of the U1 snRNP, thus suggesting that these autoantibodies may inhibit nuclear editing of RNA transcripts. The above findings, although provocative, may have limited importance in the pathologic process of SLE if the autoantibodies cannot penetrate living cells. Although some investigators have offered data suggesting penetration of cells by anti-RNP antibodies attached first via Fc surface receptors, these findings remain controversial, and the bulk of evidence so far indicates that such autoantibodies do not internalize within living cells so as to interfere with specific metabolic processes.

Rheumatoid arthritis, another major autoimmune disease, is characterized by the presence in serum of autoantibodies directed against the Fc portion of IgG. Such autoantibodies, usually of IgM or IgG isotype, combine with IgG to form immune complexes that are considered to participate in the associated synovitis and vasculitis via activation of the complement cascade and attraction of polymorphonuclear cells to the sites of their deposition (see Chapter 21). Some rheumatoid factors cross-react with other autoantigens such as DNA, histones, and cytoskeletal elements. Rheumatoid factor production, however, is not restricted to autoimmune diseases; recent findings suggest that it is a component of normal immune responses, not necessarily pathogenic, and, in fact, may play a significant role in normal immunoregulation and homeostasis.

Sjögren's syndrome is characterized by antibodies to 2 protein-RNA complexes termed SS-A/Ro and SS-B/La (70% and 50% incidence, respectively). Recent observations suggest that antibodies to SS-A/Ro are strongly associated with congenital complete heart block in infants with neonatal lupus caused by transplacental passage of such autoantibodies from the mother to the fetus. Therefore, maternal antibody to SS-A/Ro might serve as a marker to identify women at risk of having an infant with congenital heart block.

In most of the above-described organ-specific and non-organ-specific autoimmune diseases, not only autoantibodies but also certain cell types such as K cells and T cells have been incriminated as primary or accessory participants in their immunopathology. Of course, as will be discussed in the following sections of this review, abnormalities of regulatory T cells have been considered as one of the primary causes of autoantibody production by B cells.

Although much is known about the immunopathologic mechanisms of autoimmune diseases, their causes are still largely unknown. However, a wealth of information has accumulated in the last few years on such important topics as diversity of the immune system, means of normal immunoregulation, and tolerance induction. Thus, one can now with some confidence design a framework upon which future work concerning such disorders will be arrayed. These aspects are reviewed below.

DIVERSITY OF IMMUNE RESPONSES

Humoral and cellular diversity of the immune system is intimately connected with the question of self-nonself discrimination. The first task of an immune system is to react against virtually any foreign substance. It is well established that an individual can produce specific antibodies to all antigenic determinants in the universe. In fact, the immune system is capable of producing specific antibodies even against all sorts of odd, artificially synthesized chemicals and molecules; in other words, the immune system appears to recognize all antigenic possibilities. Simultaneously with acquisition of immune responsiveness, the immune system must fulfill a second requirement—that it exhibit no or minimal reactivity against self antigens. How such an enormous diversity of immune responses develops accompanied by a very re-

stricted responsiveness to self is not known, although several recent findings have greatly expanded our knowledge in this respect.

Hypothetically, the great diversity of self determinants is what dictates how large the immune repertoire must be. If the repertoire is limited, the possibility for reactivity to self via cross-reactions is high. If the repertoire is large, the possibility for cross-reactivity against self is greatly reduced. Two major theories offer genetic explanations for the enormous diversity of immunoglobulin (Ig) heavy (H) and light (L) chain variable (V) regions, which determine the antigen-combining sites of B cells. The **germ line theory,** in its most extreme form, postulates that for every V region there must exist a different gene in the germ line. In contrast, the **somatic mutation theory** proposes that a small number of germ line genes diversify either by point mutations or by recombination events during the differentiation of lymphocytes to create the antibody repertoire de novo in each individual. Recent studies by DNA cloning of human and murine cells containing genes for immunoglobulins (see Chapter 5) strongly indicate that both germ line and somatically mutated genes contribute to antibody diversity. Thus, it has been clearly demonstrated that although a large number of germ line antibody genes exist, further expansion of antibody repertoire occurs via somatic recombinations and rearrangements of separate segments of DNA coding for particular portions of the antibody molecule (V, variable; J, joining; D, diversity; C, constant). Molecular cloning techniques have shown that the haploid germ line genome contains a cluster of 200–500 different V_L–V_H segments, a cluster of about 12 D_H segments, a cluster of 4 functional J_H and 4 functional J_κ segments, and a cluster of 8 copies of C_H segments, one each for the 8 immunoglobulin classes or subclasses. The V_H genes have been classed into 9 subfamilies, each composed of 4–50 or more members. Members of a given subfamily exhibit greater than 80% homology at the nucleic acid level. These multiple gene segments scattered along the chromosome of a germ line genome assemble during the development of B lymphocytes and form a complete immunoglobulin gene. By further substitutions and mutations occurring during the evolution of an immune response, especially in V regions of IgG and IgA isotypes, and far less, if at all, in IgM isotype, a vast array of antibodies can be generated. Thereby, the germ line diversity of 10^4–10^5 antibody specificities is expanded to a total diversity of well over a million antibodies. Thus, through a large number of germ line genes, somatic mutations, and selection of useful variant lymphocytes that react against foreign antigens, great diversity is generated. It is unclear at what stage of B cell differentiation—from stem cell to mature B cell—commitment to an ultimate antibody specificity occurs. However, central to understanding B cell expression is the fact that most B cells are relatively short-lived (a few days), and thus the repertoire is generated over and over again, presumably from stem cells within the marrow of mature individuals. It seems that lymphocytes are constantly "learning," in an evolutionary sense, throughout the life of their host, but this accumulated experience is lost when the animal or a particular clonotype dies, and the lymphocytes of the next generation of vertebrates must start again from the baseline "knowledge" of inherited V genes.

In contrast to B cells, until recently very little was known about the mechanisms by which T cells diversify. Initial experiments in murine hemopoietic radiation chimeras strongly suggested that the major organ in which T cells differentiate and diversify is the thymus, with the major histocompatibility complex (MHC) expressed in this organ playing an important role in these processes. Ontogenic studies with molecular and immunologic techniques have recently shown that T cell antigen receptors are first expressed within the murine thymus around days 16–17 of gestation. Furthermore, molecular studies have clearly shown that the immunoglobulin gene segments are not encoding the T cell antigen receptor. However, extensive similarities in primary and secondary structure of the T cell receptor and immunoglobulin polypeptide V, D, J, and C regions were observed. Furthermore, it has been demonstrated that the clonally distributed T cell antigen recognition structures are disulfide-bridged heterodimers composed of a 49,000- to 43,000-MW α chain and a 43,000- to 40,000-MW β chain. Approximately 30,000–40,000 such sites are present per T cell. The known murine V_β gene segments represent at least 20–30 subfamilies, the majority with only one member, one with 2 members, and one with 3 members. The V_α gene segments are derived from at least 11 subfamilies, each containing 1–10 members for a total of more than 100 members. There are 2 closely linked C_β genes, $C_{\beta 1}$ and $C_{\beta 2}$, each associated with a cluster of six J_β and one D_β gene segments. No apparent functional significance for the presence of two C_β isotypes has been identified, since either one can be found in cloned cytotoxic and helper murine T cell lines. In contrast to C_β genes, there is only one C_α gene associated with a large number (> 50) of J_α segments. A third T cell receptor gene, the so-called γ chain gene, has also been identified, but its expression and function have yet to be determined. The organization of the human T cell antigen receptor genes appears to be similar to that of the murine counterparts. Like the B cell antigen receptor immunoglobulin, diversity of the T cell antigen receptor thus appears to be generated by a large number of germ line genes as well as by combinatorial joining phenomena. However, in contrast to B cells, somatic mutations appear to play little (if any) role in the diversity of the T cell antigen receptors.

FORMS OF NORMAL AUTORECOGNITION & OF POSITIVE AUTOIMMUNITY

Recognition of Self MHC by T Cells

Numerous studies on colonial marine forms and

flowering plants as well as on more sophisticated and diversified higher animals have demonstrated that self recognition mediated by cell surface receptors is a fundamental biologic process concerned with many types of developmental and differentiation events. Examples are the lectinlike cell surface molecules of the cellular slime mold *Dictyostelium discoideum* that determine cellular cohesiveness, the specific cell surface molecules of simple metazoa and of colonial tunicates that allow formation of colonies, and the cellular receptors on vertebrate embryonic cells that allow appropriate cells to aggregate into tissues and organs. Such self recognition may occur between cells having identical receptors (like-like interactions), complementary receptors (lock and key interactions), or receptors interacting via a linker molecule.

It has now been demonstrated beyond any doubt that certain elements of the vertebrate immune system preserve and express throughout life such a self recognition capacity, which apparently is essential for the normal function and diversification of the immune system. This conclusion is now a fundamental tenet of immunologic theory. The initial finding for most antigens was that collaboration between helper T cells and precursors of effector cells (B cells, cytotoxic T cells, suppressor T cells) is required for antigen-driven differentiation into mature effector cells. In addition, helper T cell differentiation begins only after association with antigen-presenting macrophages, B cells, or other antigen-expressing cells. In contrast, B cells and suppressor T cells can proliferate after contact with free soluble antigen. Significantly, in murine humoral responses, T cell help is initiated and delivered only if the T cells, B cells, and antigen-presenting cells such as macrophages are compatible at the MHC, more specifically at the I region (class II MHC antigens). Complete disclosure of the MHC's role and of self recognition in immune responses awaited discovery that T cell-mediated immunity and efficient killing of virus- or hapten-modified cellular targets also required identity of the effector cytotoxic T cells and target cells at the MHC—more specifically, at the K or D region (class I MHC antigens) of the murine H-2 (A and B for HLA). This phenomenon, applicable to both helper and cytotoxic T cells, was designated the **MHC restriction phenomenon** and was found to be operative in many species, including humans, and in vitro as well as in vivo. The restriction and interaction of T cells with self MHC-bearing stimulator cells results not from a like-like interaction between T cells and stimulator cells but from a true T cell antiself receptor.

A key question raised by MHC restriction concerns the nature of the T cell receptor. Two models have been proposed to explain the dual specificity of subsets of T cells for antigen and self MHC products (Fig 11–1): (1) The **2 recognition sites model** expresses the view that helper and cytotoxic T cells possess 2 separate recognition sites that are specific for 2 separate antigens on antigen-presenting cells or target cells, respectively; one receptor site binds to the restricting self MHC, and the other binds to the cell surface-associated non-MHC antigen. (2) The **single recognition site model** is based on the assumption that T cells express a single receptor site that is specific for a single neoantigenic determinant formed when the self MHC complexes with the foreign antigen on antigen-presenting cells or target cells. Molecular and other studies appear to favor the single recognition site model over the 2 recognition sites model.

It is not known why T cells are MHC-restricted and need to recognize self for optimum participation in humoral and cellular responses. This trait may distinguish them from B cells or antibodies, which do not express such a restriction. Probably this dual recognition is required for efficient stimulation of T cells that might be endowed with low-affinity receptors for antigen only. Such dual recognition would appear to be advantageous for survival of the species. For example, cytotoxic T cells seem to be essential for recovery from some acute viral infections. By recognizing and lysing virus-infected cells displaying viral antigens plus self MHC antigens, before assembly of progeny virus particles, these T cells limit viral multiplication. If their antigen receptors bound avidly to free antigen (viral) molecules, these receptors would be inhibited in binding to foreign antigen on an infected cell's surface, thus reducing the cytotoxic T cell's antiviral function. Evolutionary pressure would therefore lead to retention of the self-recognition capability in cytotoxic T cells, which presumably evolved from a self-recognition system that existed before the appearance of adaptive immune responses. Whatever the models and explanations, it is now clear that recognition by T cells of self MHC along with an antigen is a prerequisite for generation of effector immune functions.

Recognition of Self Immunoglobulin V Region Determinants & the Idiotype-Anti-idiotype Network

As summarized above, responses of T cells to self MHC antigens play an essential role in *initiating* both humoral and cellular immune responses. We shall see now that responses to self may also play a role in *regulating* these immune responses.

Clearly, the immune system interacts with antigenic determinants, also called **epitopes,** via antigen-combining sites present in the hypervariable regions of V domains within immunoglobulin molecules either on the surfaces of or secreted by lymphocytes. Important discoveries clearly demonstrate that an antibody molecule has a dual character, acting to recognize a given antigen and, in turn, itself becoming immunogenic, even in the animal that produces this antibody. The variable regions of a given immunoglobulin alone can act as antigenic determinant to generate another set of antibodies which recognize the uniqueness of that immunoglobulin as distinct from antibodies of different specificities. Sets of antigenic or epitopic determinants of immunoglobulin V domains were termed **idiotypes,** and the antibodies elicited against them were termed **anti-idiotypes.** Each single idiotypic epi-

tope located on different portions of the V region was called an **idiotope.** An anti-idiotope antibody does not react with the entire array of idiotypic determinants of an immunoglobulin molecule but only with a single determinant, the idiotope. However, anti-idiotypic antibodies of a single specificity may be represented in different immunoglobulin classes. Idiotypic determinants have been described that are on V_L alone, V_H alone, or both, involving either antigen-binding sites (complementarity determining regions, CDR) or noncombining sites (framework regions, FR) of the V domains (Fig 11–2). Idiotypes representing antigenic differences of immunoglobulin molecules at the V region differ from **allotypes,** which result from inherited variations (polymorphism) in the genes coding for certain amino acid sequences in the constant (C) region of immunoglobulin molecules, and from **isotypes,** which depict the different C regions found on immunoglobulin molecules (C_μ, C_δ, $C_{\gamma 3}$, $C_{\gamma 1}$, $C_{\gamma 2b}$, $C_{\gamma 2a}$, C_ε, C_α). Both B cells and T cells, as well as their soluble products (antibodies, antigen-specific T cell-derived helper and suppressor factors), express idiotypic determinants. Moreover, in accord with the clonal selection theory of Burnet (see below), the idiotype of immunoglobulin secreted by an antibody-forming B cell is the same as that of the cell surface immunoglobulin receptors for antigen. The number of idiotypes an individual possesses is apparently as large as its range of antibody specificities or repertoire.

The responses of animals to most antigens involve several clones of reactive cells producing antibodies that have many idiotypic specificities or antigenic differences in the V region. However, after antigenic challenge, only a few clones of lymphocytes expand, resulting in the expression of a dominant idiotype. Idiotypic cross-reactions in inbred animals such as mice are not uncommon, especially among antibodies directed against relatively simple antigenic determinants. In contrast, most outbred animals and humans infrequently express cross-reactive idiotypes consistent with the broadly heterogeneous regions that can combine with the different antigenic determinants present on a complex antigen. Nevertheless, even in outbred species, cross-reactive idiotypes are occasionally present as a result either of inheritance of antibody genes among related individuals or of preservation and sharing of certain germ line genes by unrelated individuals within the species.

The dual characteristics of antibody molecules and

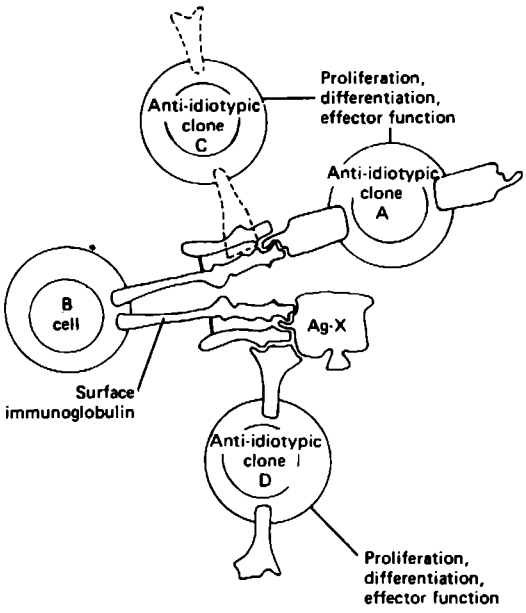

Figure 11–2. Specificity of anti-idiotypic antibodies. In this model, a B cell clone is the predominant clone reacting to an antigenic determinant on antigen X (Ag-X). Having expanded to the point that its antigen-binding receptors reach an immunogenic level, the B clone stimulates 3 separate lymphocyte clones each of which possesses surface receptors that recognize idiotypic determinants on the immunoglobulins expressed by the B clone and its progeny cells. Anti-idiotype clone A recognizes idiotypic determinants within the antibody combining site, whereas clone C recognizes H chaln idiotypic determinants outside the combining site, and clone D recognizes L chain idiotypic determinants in combination with determinants on antigen X. Each of these anti-idiotype clones could be in the T or B cell series, and the cells or their products could express helper or suppressor functions, leading (respectively) to idiotype-specific augmentation (positive feedback) or inhibition (negative feedback) of this immune response. In turn, as each anti-idiotype expands to the point that its antigen-specific receptors reach immunogenic levels, anti-(anti-idiotypic) responses of a positive and negative type could be induced. (Modified and reproduced, with permission, from Hood LE, Weissman IL, Wood WB: Page 38, Fig 1–29, in: *Immunology*. Benjamin/Cummings, 1978.)

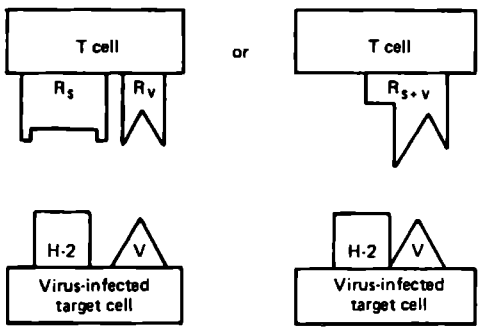

Figure 11–1. The one-receptor, 2-receptor hypothesis for cytotoxic and helper T cell recognition. It is still not clear whether the T cell expresses one receptor specific for self H-2 (R_s) and another that recognizes nonself antigen (in the illustration, viral antigen R_v), or a single receptor that recognizes a complex of self H-2 and non-H-2 antigens (in the illustration, viral [V] antigens). (Reproduced, with permission, from Doherty PC, Bennink JR: Monitoring the integrity of self: Biology of MHC-restriction of virus-immune T cells. *Fed Proc* 1981;40:218.)

the apparent presence within an individual's repertoire of V genes with specificities for other V region products stimulated Jerne to propose in 1974 that autoimmune responses to self idiotypes might form the basis of immunoregulatory network systems in which homeostasis is preserved through a functional assembly of idiotype-anti-idiotype interactions. According to this model, an antigen induces the production of an antibody (Ab_1) characterized by its idiotype (Id_1). In turn, the latter stimulates the synthesis of an anti-idiotypic antibody (anti-Id_1 or Ab_2) bearing the idiotype Id_2 that can trigger the production of anti- (anti-idiotypic) antibody (anti-Id_2 or Ab_3), and so on. Initial models of the network system suggested that the network was open-ended and of unlimited extent, whereas more recent studies tend to support a circular configuration of limited sets of idiotypes and anti-idiotypes. According to network theories, for every **paratope** (antigen combining site of an antibody molecule), a complementary fitting idiotope on another antibody molecule can be found, and vice versa. Such an idiotope must be stereochemically similar (3-dimensional) to the epitope on the antigen against which the antibody was originally directed. Jerne calls the subset of immunoglobulin molecules that contain these idiotypes the **internal image set;** Lindenmann calls them **homobodies.**

Jerne assumes that such an idiotypic-anti-idiotypic network is functional, which means that its regulation should account for the various modes of the immune response (steady state, enhancement, suppression). One must infer that suppressive interactions dominate stimulatory ones in order to avoid the aberrant proliferation of clones. Moreover, before antigenic challenge, the system is in a virgin state that can be regarded as a stable reference state. Upon the introduction of antigens, macroscopic perturbation occurs and drives the system toward a new steady state characterized by immune memory or tolerance.

The idiotype network concept is now widely accepted because of a rather impressive accumulation of data in support of its existence and functional importance. Thus, auto-anti-idiotypic antibodies as well as operational suppressive and enhancing networks have been described. Auto-anti-idiotypic antibodies have been detected in mice immunized with T cell-independent antigens such as phosphorylcholine, bacterial levan, and trinitrophenyl (TNP)-Ficoll. Antibody titers and numbers of specific antibody-secreting plaque-forming cells apparently decrease while the amount of auto-anti-idiotypic antibodies increases, which seems to indicate that auto-anti-idiotypic antibodies exert a negative feedback on expression of the immune response. Moreover, in agreement with predictions made by the network theory, when experimentally produced anti-idiotypic antibodies are administered passively to animals, suppression or enhancement of the relevant idiotype may ensue. This is accomplished via the interaction of sets of B cells, helper and suppressor T cells bearing complementary idiotypic and anti-idiotypic determinants, identical idiotypic determinants through an antigen bridge or an anti-idiotype bridge, or identical anti-idiotypic determinants through an idiotype bridge. For suppressive effects, the following hypothetical sequence of events could take place (Fig 11–3). First, antigens (in the illustrated instance, a T cell-independent antigen) induce proliferation of B lymphocytes, whose receptors are characterized by Id_1. In a first step, precommitted precursor pre-B_1 cells differentiate into mature B_1 lymphocytes. The latter proliferate and differentiate into plasma cells secreting an antibody (Ab_1) characterized by Id_1. Ab_1 recognizes and eliminates the antigen. Its idiotype (Id_1) stimulates the proliferation of anti-idiotypic (anti-Id_1) B_2 cells, which differentiate from the precursor pre-B_2. Presumably, this step is dependent on T cells that carry anti-Id_1 receptors, since it has been established that an immune response to an immunoglobulin molecule requires helper T cells. The B_2 cells differentiate into plasma cells secreting αId_1 (Ab_2) antibodies, which act negatively on the Id_1-bearing B_1 cells. Anti-idiotypic antibodies, upon attaining concentrations above a critical threshold, can also exert negative influences on these idiotype-expressing cells by engaging subsets of suppressor T cells that express the relevant anti-idiotype. An intermediate helper T cell whose receptor would express Id_1 idiotype may be necessary for interaction with anti-idiotypic antibodies and induction of anti-Id_1-bearing suppressor T cells. By studying idiotype-induced suppres-

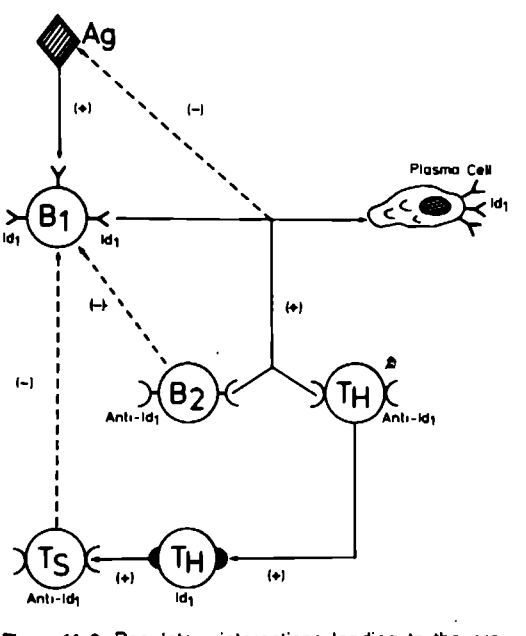

Figure 11–3. Regulatory interactions leading to the production of autoanti-idiotypic antibodies (anti-Id_1) that suppress the proliferation and differentiation of Id_{1+} clones in the case of a T cell-independent response. Suppression can be achieved with or without the participation of suppressor T cells (see text). (+), Activation; (−), inhibition.

sion in mice, suppressor T cells have been subdivided into 3 populations. The first population (Ts_1) is Lyt 1^+, bears I-J and idiotypic determinants, and functions in a non-H-2(?) or V_H-restrictive manner during the induction phase of the immune response. The Ts_1 population secretes a factor (TsF_1) which, together with antigen, induces a second complementary population of suppressor cells (Ts_2) that bear Lyt 2 alloantigen, I-J determinants, and anti-idiotypic receptors. The latter population functions in an H-2 and V_H-restrictive manner during the effector phase of the immune response in previously primed animals. However, this second population does not contain the final suppressor T cells. The available data suggest that the Ts_2 cells via a soluble factor (TsF_2) activate a third population of Lyt 2, I-J, and idiotype-bearing T cells (Ts_3), the possible final suppressors in the immune recipient.

Anti-idiotypic antibodies can also induce enhancement of immune responses instead of suppression. This can result either (1) from the stimulation of idiotype-bearing helper T cells that then induce idiotype-bearing B cells, or (2) from the elimination of specific suppressor T cells. In this last instance, anti-idiotypic antibodies (anti-Id_1, Ab_2) might induce the production of anti-anti-idiotypic antibody (anti-Id_2, Ab_3), which is assumed to suppress the production of anti-Id_1 antibody by B cells on the one hand and to eliminate anti-Id_1-bearing suppressor cells on the other.

Thus, various experimental models seem to indicate that idiotypic interactions, essentially autoimmune in nature, can play an important role in functional regulatory circuits of the immune system and in promoting communications between T and B cells. The system emerges as a self-contained, highly organized network of complementary T and B cell surface-bound and soluble idiotypes that is constantly engaged in recognition of self. If one agrees that idiotypes may sterically represent mirror images of antigens, it would be reasonable to suppose that the immune system accepts the structural diversity in the universe as nothing new or strange since it "sees" these structures continuously in its complementary circuits. According to this somewhat extreme but provocative view, "foreign" determinants are in fact never foreign, and consequently, challenge with antigens is an epiphenomenon that does not set in motion any novel element in the system but only perturbs it until it reaches a new steady state.

THEORIES OF TOLERANCE INDUCTION

What has been said makes it clear that vertebrates have all the genetic information necessary to respond immunologically against self and nonself constituents and the ability to induce and regulate their immunologic apparatus via self recognition processes. Despite these conclusions, however, it is well established that the immune system of a normal individual is in general phenotypically tolerant to self.

Early studies demonstrated that tolerance to self is acquired through an active process that involves a direct contact between self components and specific antigen-reactive cells. For example, removing the hypophysis from a tree frog during early life (tadpole), allowing the gland to differentiate in isolation from its donor, and finally, transplanting the organ back into the mature donor results in rejection instead of acceptance. Moreover, animals genetically deficient in certain proteins still make antibodies when injected with the proteins. After studying responsiveness to exogenous antigens in experimental animals—and assuming that responses against them are controlled by mechanisms similar to those for endogenous antigens—investigators have advanced several hypotheses to explain the apparent unresponsiveness to self by an individual possessing the genetic information to do so. There are 2 types of tolerance: central and peripheral. In central forms, no antibody is produced at all after antigenic challenge, since the responsible immunocytes are missing (clonal deletion) or are silenced directly (clonal anergy). In peripheral forms, some antibody is initially produced; but then, because of the expression of suppressive mechanisms (suppressor T cells, antibodies, anti-idiotypes, and immune complexes), antibody production diminishes greatly or ceases entirely.

The first serious attempt to explain how unresponsiveness to self might be acquired was undertaken by Burnet as a corollary to his clonal selection hypothesis. Burnet was the first to introduce the concept of the immunologic **clone**, a subset of immunocytes all having identical receptors for antigen. Burnet's **clonal selection** theory of acquired immunity proposed that virgin clones of immunocytes circulate in the body awaiting contact with their specific antigens, after which they undergo blast transformation and divide repeatedly to produce thousands of descendant cells of the same specificity. This concept followed Jerne's postulation that antibody molecules are not fashioned on a template formed by an invading antigen but are preformed, waiting to be selected by their complementary antigen. In other words, antibodies are made before the exposure to antigens, and their combining site specificity is solely determined by structural genes upon which antigens have no influence other than inducing proliferation of the clone that expresses the specific idiotype or V domain for that antigen.

In any event, Burnet proposed that immune tolerance to self antigens is subserved by a mechanism that causes fetal immunocytes to be deleted by contact with their specific autoantigen (**clonal deletion**) (Fig 11–4). This proposition owes its origin to the work of Owen, who first demonstrated that contact with foreign antigenic substances during early life resulted in immunologic tolerance. He observed that mature dizygotic twin cows tolerated each other's body tissues in that they did not reject mutual grafts. Undoubtedly, the tolerance resulted from embryonic parabiosis in which blood was exchanged between the twins. Subsequently, Billingham and Medawar found that

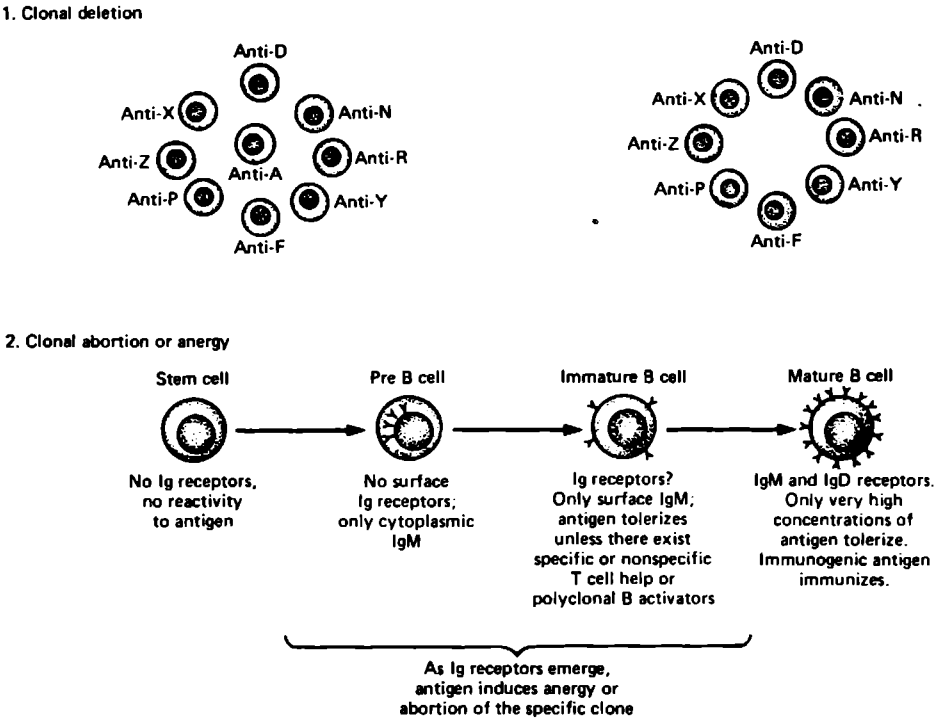

Figure 11–4. The clonal deletion and clonal abortion (anergy) theories of B lymphocyte tolerance. (Modified and reproduced, with permission, from Nossal GJV, Pike BL: Page 136 in: *Immunology 80: Progress in Immunology*. Academic Press, 1980.)

adult mice of an inbred strain tolerated skin grafts of a second inbred strain if, as newborns, animals of the first strain were injected with replicating cells of the second strain. For the development of autoimmune diseases, Burnet suggested that precursor lymphocytes committed to nonself but related to self antigens mutate during their multiplication and accidentally make lymphocytes reactive to self.

The above theory was later redefined and expanded by Nossal and associates, who coined the terms "clonal abortion," **"clonal anergy,"** "clonal silencing," and "clonal purging"—all to describe the same event. Nossal proposed that at some stage in their differentiation from stem cells to mature antibody-forming precursor cells, B lymphocytes go through a phase during which contact with even low doses of antigen (whether endogenous or exogenous) induces tolerance and not immunity (Fig 11–4). This phase is referred to as the **tolerance-sensitive** or **obligatory paralyzable phase.** Further experiments showed that the tolerance-inducing encounter between antigen and immature B cells did not lead to death of the tolerant cells. In other words, the immature B cells received and stored some negative signal without having been eliminated. Given that an animal can have antigen-binding B cells incapable of reacting to antigen and present in a functionally silent state, the process whereby such tolerance is induced was more accurately redefined as "clonal anergy" than "clonal abortion" or "clonal deletion." Since the initial description of these findings, additional studies have clearly demonstrated the ease of inducing tolerance in neonatal spleen cells and bone marrow cells of neonates and adults with a concomitant resistance of tolerance induction in adult splenocytes. Significantly, spleens of adult animals contain a minor subset of immature B cells that can be tolerized as easily as neonatal B cells. This finding suggests that the ease of tolerance induction is not a unique property of neonatal cells but of immature B cells, in general, which are continuously produced throughout life.

Another major mechanism proposed for centrally induced tolerance is that of antigen- or ligand-induced inactivation of immune responsiveness (Fig 11–5). The term **ligand-induced inactivation** is used in a broadly descriptive sense to indicate the loss of reactivity in a population of specific immunocompetent lymphocytes as a direct consequence of interaction between antigenic determinants and the cell surface receptors binding such determinants under circumstances or conditions that are either particularly unfavorable to triggering or are particularly favorable to inactivation of the cell. Monomeric antigens could be incapable of signaling the B cell, in which case, at high molarity, they could occupy the B or T cells' antigen receptors so as to prevent macrophage-associated or other immunogenic forms of antigen from gaining

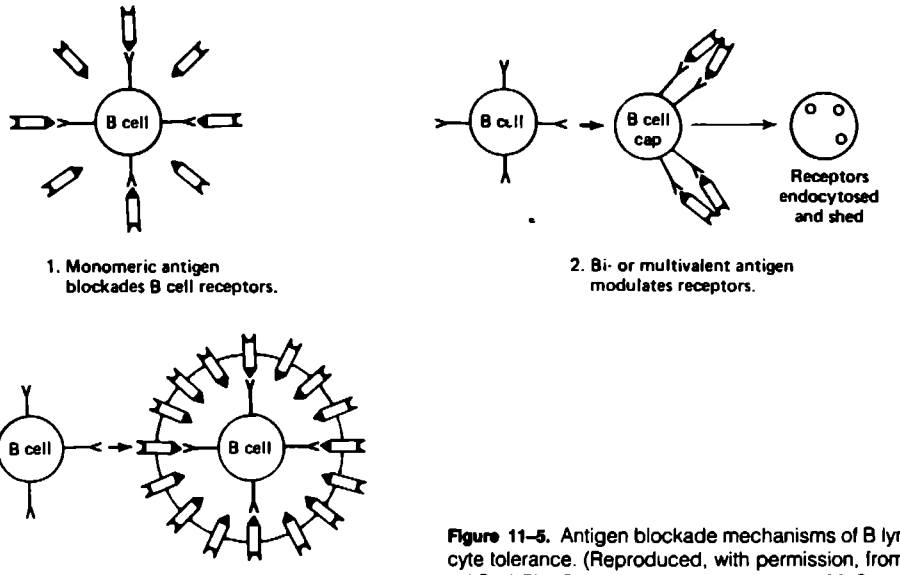

Figure 11–5. Antigen blockade mechanisms of B lymphocyte tolerance. (Reproduced, with permission, from Nossal GJV, Pike BL: Page 136 in: *Immunology 80: Progress in Immunology*. Academic Press, 1980.)

access to these cells. It is perhaps relevant that aggregate-free forms of antigens are much more tolerogenic than the aggregated forms. Bivalent to multivalent antigens could, on the other hand, induce tolerance by depriving the cell of its antigen receptors through the patching-capping-endocytosis cycle (a process called modulation). A final possibility is that high concentrations of certain multivalent antigens—especially those which are linear with repetitive units—can immobilize antigen receptors, "freezing" the membrane and inhibiting transmission of stimulatory signals.

Although clonal abortion or anergy as well as antigen blockade may be significant means by which tolerance to self is achieved, additional studies suggest that the absence of autoimmune reactions may result, at least in part, from continuous and active suppression exerted peripherally by subsets of T cells, the so-called **suppressor T cells** (Fig 11–6). In such instances, suppressor T cells may carry anti-idiotypic determinants for interacting with and silencing a B cell that carries the complementary idiotype, or they may antagonize the action of a helper T lymphocyte as outlined in previous sections and in subsequent ones.

Although the role of suppressor cells in tolerance induction and regulation of responses against conventional antigens, transplantation antigens, allotypes, and idiotypes is well documented, the importance of these cells in inducing tolerance to autoantigens and in preventing autoimmune responses remains uncertain. However, recent observations suggest that this mechanism may be operative in immunologic self-tolerance. Thus, the existence of autoreactive T helper and B cells in normal animals provides a priori agreement in favor of a role for active suppressor mechanisms in regulating anti-self responses. Furthermore, it was recently demonstrated that depletion of suppressor T cells in vitro by exposure to anti-I-J antibodies (I-J determinants are present on suppressor but not helper T cells) provoked a highly significant spontaneous autoantibody response and increased autologous mixed lymphocyte reaction. Finally, suppressor T cells for autocytotoxicity could be demonstrated in vitro, and newborn animals appear to have an excessive number of suppressor T cells in the thymus and spleen.

Other peripheral means of tolerance induction include circumstances in which the antibody alone or in the form of **immune complexes** in antibody excess may act suppressively relative to the ongoing response. Suggested mechanisms of antibody-mediated or immune complex-mediated suppression include (1) antigen shielding or masking by antibody, resulting in blockade of antigen recognition by antigen receptor-bearing lymphocytes; (2) activation of IgG Fc receptor-bearing suppressor T cells, with subsequent release of soluble factors that suppress helper T cells; and (3) direct stimulation of B cells with Fc receptors to secrete soluble suppressor factors. Although some forms of antibody-induced suppression require the Fc portion of the molecule, others do not. Some investigators suggest that suppressive effects exerted by high concentrations of antibody are independent of the Fc portion, whereas those exerted by low concentrations are Fc-dependent. Antibody or immune complexes may also serve as "blocking factors" that impede an effective immune response to such cell-associated antigens as those on tumor cells. The premise is that such a response requires access of cytolytic T cells to antigenic determinants on the cell surface. However, stimulation of humoral immune responses and subsequent complexing of antibodies with antigen may re-

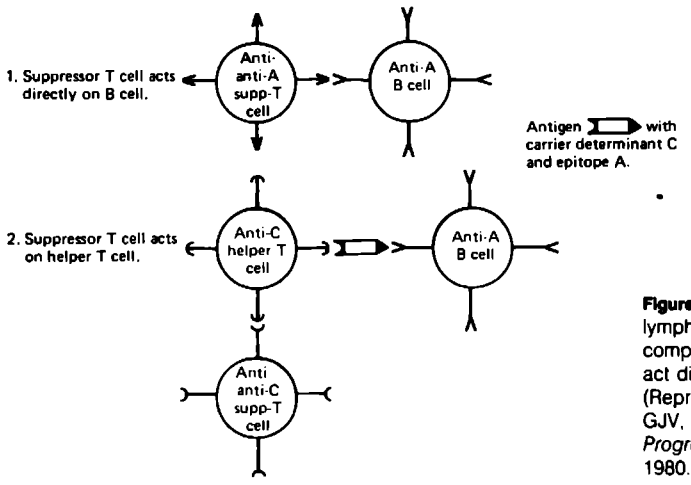

Figure 11–8. Suppressor mechanisms of B lymphocyte tolerance. Suppressor T cells with complementary anti-idiotypic determinants may act directly either on B cells or helper T cells. (Reproduced, with permission, from Nossal GJV, Pike BL: Page 136 in: *Immunology 80: Progress in Immunology*. Academic Press, 1980.)

sult either in masking of the antigenic determinants or occupation of the antigen receptors on the cytolytic cells, blocking these cells' access to and preventing direct interaction with the target cell. Interestingly, autologous anti-idiotypic responses of mice to cytotoxic lymphocytes with specificity for tumor antigens were found to suppress tumor regression, presumably via occupation of the antigen receptors on cytotoxic cells.

As can be seen from the above account, a variety of means exist for induction of tolerance or unresponsiveness. Some inhibit immune responses centrally by paralyzing or silencing the responding cells, others by acting peripherally via suppressor cells or antibodies. Central forms of tolerance appear to be more compatible with the expected mechanisms for induction of tolerance to self. However, the mechanisms described above should not be regarded as mutually exclusive. Indeed, it seems certain that different forms of the tolerant state result from different mechanisms, and a diversity of mechanisms may be operative simultaneously. Because self antigens occur in diverse forms and concentrations, undoubtedly the same is true for physiologic self-tolerance.

ETIOLOGY & PATHOGENESIS OF AUTOIMMUNE DISEASES

Many theories and mechanisms have been proposed for the generation of autoimmune responses (Table 11–2). Their theoretic bases and controversies concerning each are presented below, along with pertinent examples and findings in humans and in appropriate experimental animals, especially murine SLE, summarized in parallel. Reviewing the available evidence, one must surmise that such spontaneous diseases have a multifactorial basis, with immunologic, genetic, virologic, hormonal, and other factors playing essential roles, each acting singly or synchronously with the others.

IMMUNOLOGIC FACTORS IN AUTOIMMUNE DISEASES

Release of Sequestered Antigens

As indicated above, for induction of unresponsiveness to self, contact between autoantigen and the immune system is required early in ontogeny. If an antigen is sequestered within an organ, thus precluding contact with the lymphoreticular system, no immunologic tolerance at the T or B cell level can be established. However, any tissue damage that exposes the antigen later in life would then provide an opportunity for autoantibody formation, since tolerance induction in adults with many mature B cells is much more difficult to achieve than in neonates, whose immature, easily tolerized B cells predominate. Autoantibody production following release of sequestered antigens has been repeatedly demonstrated, eg, autoantibody formation against sperm after vasectomy, against the crystalline lens after eye injury, against heart muscle antigens after myocardial infarct, etc. In most of these instances, the autoimmune response is transient and

Table 11–2. Theories of origin of autoimmunity.

Release of sequestered antigens
Diminished suppressor T cell function
Enhanced helper T cell activity, T cell bypass
Thymic defects
Presence of abnormal clones, defects in tolerance induction
Polyclonal B cell activation
Refractoriness of B cells to suppressor messages
Defects in macrophages
Stem cell defects
Defects in the idiotype–anti-idiotype network
Abnormal genes: Immune response genes, immunoglobulin genes, T cell receptor genes
Ectopic Ia expression, altered Ia
Viral factors
Hormonal factors

probably disappears before clinical symptoms are generated. Progressive autoimmune disease appears to require persistent antigen that is presented in an immunogenic form.

Diminished Suppressor T Cell Function

Immune responses are normally down-regulated by complex interactions between immune response-helping and immune response-suppressing cells and their soluble products. It follows, therefore, that loss of a given autoantigen-specific suppressor T cell subset or nonspecific loss of this class of cells could result in the spontaneous appearance of autoantibodies. Antigen-nonspecific suppressor T cells can be identified numerically by the expression of certain surface alloantigens detected by polyclonal or monoclonal antibodies or by expression of surface Fc receptors for IgG. Functionally, these suppressors are marked by their ability to release soluble products after stimulation with concanavalin A (Con A), which suppresses B mitogen-induced polyclonal activation and immunoglobulin secretion or allogeneic mitogenic responses. With these methods, numerical or functional abnormalities of suppressor T cells have been noted in a variety of organ-specific and generalized autoimmune disorders. However, the validity of ascribing reduced suppressor T cell number or function assessed by the above procedures as causes of organ-specific autoimmune diseases must be questioned for the following reasons: (1) It is very difficult to imagine how a generalized suppressor T cell defect could be expressed as an organ-specific autoimmune disease; and (2) it is equally difficult to imagine how elimination, reduction, or dysfunction of a very minor subset of suppressor T cells that reduces the activity of immunocyte clones responsive to a specific autoantigen could be expressed in the total compartment of suppressor T cells which are enumerated with monoclonal antibodies or assessed functionally by Con A stimulation. Although more acceptable on theoretic grounds, the numerical or functional diminution of total suppressor T cells in generalized autoimmune diseases, such as SLE, involving many autoimmune responses also remains controversial. Generally, such subjects have significant lymphopenia and decreases in absolute and relative numbers of T cells. T cell functional studies in humans with SLE reveal, in most cases, impaired delayed hypersensitivity to various antigens in addition to decreased proliferative responses to T cell mitogens and to autologous (syngeneic mixed lymphocyte response) and allogeneic stimulator cells. The severity of some of these impairments was thought to correlate positively with disease activity. Moreover, the subject of repeated claims is that T cells from patients with active SLE cannot generate antigen-nonspecific suppressor signals. This was considered to represent an inherent defect of T suppressor cells, since B cells from such patients show normal responses to suppressor activity generated by normal T cells. A related assertion is that antilymphocyte antibodies present in sera of most SLE patients have preferential reactivity with suppressor T cell subsets. The primary importance of these findings remains questionable, since most patients included in these studies are at an advanced stage of their disease and are under treatment with a variety of anti-inflammatory drugs, such as corticosteroids, and of cytostatic agents that profoundly affect the immune system. Recent studies have failed to confirm some of the above findings in that polyclonal suppressor T cell activity of the SLE patients examined was within normal limits, and no preferential reactivity of antilymphocyte antibodies with any particular immunocyte type was observed.

Similar to the studies in humans with systemic lupus erythematosus, initial studies in the spontaneous SLE model of NZB and (NZB × NZW)F_1 (New Zealand [NZ] strains) mice suggested lack of antigen-nonspecific suppressor T cell function with age and the parallel appearance of disease; the Lyt 123^+ T cell subset responsible for feedback suppression was reported to be absent or malfunctioning in NZ mice, whereas in BXSB mice the claim was made that Lyt 1^+ cells were unable to induce Lyt 123^+ cells, and in MRL/l mice, Lyt 1^+ cells were insensitive to suppressor effects by Lyt 123^+ cells. However, subsequent studies showed no apparent defect in antigen-nonspecific (Con A-induced) or exogenous antigen-specific suppressor T cell function in the susceptible NZ mice or the 2 newly described SLE murine strains, BXSB and MRL. The origins and the histologic, serologic, and cellular characteristics of these SLE-prone strains of mice are shown in Table 11–3. Additional studies in these and other murine strains have brought into question the primary importance of natural thymocytotoxic antibodies in the development of murine SLE, since not all of the above 3 afflicted strains have natural thymocytotoxic antibodies in their sera. Moreover, natural thymocytotoxic antibodies are found in some recombinant lines of NZB with normal strains in the absence of other types of autoantibodies and vice versa, and some strains with natural thymocytotoxic antibodies in their sera do not have detectable disease. Therefore, the above studies cast doubt that a *generalized* defect of suppressor T cells or the presence of natural thymocytotoxic antibodies causes autoimmunity. However, these findings still do not exclude abnormalities of specific subsets of suppressor T cells that control responses to autoantigens, nor do they exclude a secondary role of anti-T cell autoantibodies in accelerating autoimmunity. Perhaps with the availability of more sophisticated techniques such as experimentally induced specific elimination in vivo of suppressor T cells and the study of autoantigen-specific suppressor T cell function as well as of idiotype-anti-idiotype regulation, clarification of the role of suppressor T cells in autoimmunity will be forthcoming.

Escape of Tolerance at the T Cell Level & Antigenic Mimicry

As stated, for most immune responses to antigens, collaboration between helper T cells and B cells is required. It has been proposed that unresponsiveness to

Table 11–3. Characteristics of SLE-prone murine strains.

1. Derivation and Genetic Markers

Strain	Derivation	H-2	Lymphocyte Surface Alloantigens	Igh-1(IgG2a) Allotype	Accelerating Gene(s)
NZB NZW	Inbred for color from stock of undefined background	$H\text{-}2^d$ $H\text{-}2^z$	Thy 1.2, Lyt 1.2, Lyt 2.2, Lyt 3.2, $Qa\text{-}1^a$, Mls^a	e e	?
BXSB	Derived from $(C57BL/6J \times SB/Le)F_1$	$H\text{-}2^b$	Thy 1.2, TL^-, Lyt 1.2, Lyt 2.2, Lyt 3.2, $Qa\text{-}1^b$	b	Y-linked
MRL	Genome = 75% LG, 13% AKR, 12% C3H, and 0.3% C57BL/6	$H\text{-}2^k$	Thy 1.2, TL^-, Lyt 1.2, Lyt 2.1, Lyt 3.1, $Qa\text{-}1^b$	J	lpr only in MRL/l (MRL/Mp-lpr/lpr) but not in MRL/n (MRL/Mp-+/+)

2. Mortality Rates

Strain	Sex	50% (months)	90% (months)
NZB	Female	16	21
	Male	17	23
NZW	Female	24	~2
	Male	25.5	~5
$(NZB \times NZW)F_1$	Female	8.5	12.8
	Male	15	19
MRL/l	Female	5	7.3
	Male	5.5	8.6
MRL/n	Female	17	23
	Male	23	27
BXSB	Female	20	24
	Male	5.5	8

3. Histoimmunopathologic Characteristics

Strain	Glomerulo-nephritis	Thymic Atrophy	Lymphoid Hyperplasia	Arteritis	Myocardial Infarction	Arthritis
NZB	+	+++	+	0	+	0
$(NZB \times NZW)F_1$	+++	+++	+	0	+	0
MRL/l	+++	+++	+++	+++	+	+++
BXSB	+++	+++	++	0	+	0

4. Serologic Characteristics

Common	Hypergammaglobulinemia, antinuclear antibodies, anti-dsDNA, anti-ssDNA, antihapten antibodies, high levels of gp70, immune complexes (DNA-anti-DNA, gp70-anti-gp70), reduced complement levels (NZB is C5-deficient).
Uncommon	Anti-Sm (MRL mice; with sensitive techniques, such antibodies can also be found in the other SLE strains), IgG and IgM rheumatoid factors and intermediate complexes (MRL/l), antierythrocyte (NZB; NZB x NZW), NTA (NZB, NZB x NZW, BXSB).

5. Surface and Functional Characteristics of Lymphoid Cells

B cells	Hyperfunction and polyclonal activation in all strains, autoimmune clones in all strains, increased number in BXSB mice only, normal ontogeny of isotype diversity in all strains, normal capping and interiorization of Ig-anti-Ig complexes, early ontogeny of TI-2 responses in NZ mice, defective antibody-mediated suppression in all strains. Hyperresponsiveness to accessory signals in NZ and BXSB, increased frequency of B cells with Lyt 1 antigen in NZ and BXSB.
T cells	Generalized suppressor function normal in all strains; T cell number (Lyt 1+) and nonspecific helper T function increased in MRL/l only; defects in interleukin-2 production and response in all strains; reduced syngeneic mixed lymphocyte response in all strains; cytotoxicity against H-2-compatible allogeneic cells in NZ mice only.
Macrophages	Increased frequency of Ia^+ macrophages in older MRL/l and NZB.
Tolerance	Defective tolerance induction to some exogenous antigens in all strains.
Thymus	Essential in MRL/l disease but not in NZ and BXSB disease.
Nonlymphoid tissues	Noncontributory to disease development in any SLE-prone strain.

self is maintained by self-tolerance at the helper T cell level. In activation of such tolerant helper T cells via nonself antigens that cross-react with self—or substitution of helper T cells with certain nonspecific factors—existing B cells not tolerant to self can be activated to produce autoantibodies. This concept derives from the work of Weigle, Chiller, and associates, who found that T and B cells have different antigen concentration requirements for induction of tolerance and that escape from a tolerant state occurs much faster at the B cell level than the T cell level. Thus, after injection of deaggregated human gamma globulin as a tolerogen, tolerance induction in the intact mouse takes 4–5 days for completion. Induction of tolerance in either thymus cells or peripheral T cells is rapid and parallels the kinetics of tolerance induced in the intact animal; peripheral B cells are only slightly slower to assume the tolerant state. Conversely, a latent period of 8–15 days follows injection of the tolerogen before tolerance is noticeable in bone marrow cells, and the tolerant state is not complete in these cells until 21 days have elapsed. Of more importance to self-tolerance is the marked kinetic difference in the spontaneous termination of tolerance in peripheral T and B cells. Peripheral T cells, like intact mice, remain tolerant for 100–150 days, although peripheral B cells return to complete competency 50–60 days after injection of tolerogen. Further studies have shown that the dose of antigen required to induce tolerance in adult thymus cells or peripheral T cells is 100–1000 times less than that required to induce tolerance in adult bone marrow cells or peripheral B cells. Therefore, when central unresponsiveness is induced with small doses of antigen, B cells remain competent or escape very fast from tolerance, whereas T cells become tolerant for a long time. On the basis of these findings, self-tolerance is apparently maintained despite the presence of self-responding B cells owing to the lack of appropriate help from the tolerant T cell partners. Accordingly, autoimmunity is then inducible either (1) by direct stimulation of autoantigen-reactive B cells with polyclonal activators (see below); (2) by circumvention of the T cell-unresponsive state to self antigens through nonspecific T cell replacing helper factors; or (3) by induction of helper T cells via altered forms of the tolerated self antigen or with antigen that cross-reacts with the tolerated self antigen. Induction of autoreactivity by certain polyclonal B cell activators is discussed below. Factors have also been isolated from activated T cells capable of causing competent B cells to proliferate and differentiate.

Termination of specific immunologic tolerance by immunization either with altered preparations of the tolerated antigen or with antigens that cross-react with the tolerated antigen has been well documented by many investigators. Thus, rabbits made tolerant to bovine serum albumin (BSA) after neonatal injection lose their tolerant state following injection of chemically altered BSA or heterologous albumins that cross-react with BSA. In this situation, the unrelated determinants on the cross-reacting albumins seem to activate T cells, permitting stimulation of B cells competent for both BSA and the unrelated determinants. Presumably, provision of a new carrier determinant for which no self tolerance has been established bypasses the tolerant T cells and induces them to collaborate with B cells not tolerant to self to produce autoantibodies. A new carrier determinant could arise through some modification of the self molecule—eg, by defects in synthesis or abnormalities in lysosomal breakdown, yielding a split product and exposing new groupings; by combination with a drug; by association with a new antigen that drugs or viruses have induced on cells; or as a result of the presence of exogenous cross-reactive antigens that provide the new carrier with the ability to provoke autoantibody formation. Incorporation of autoantigens into Freund's complete adjuvant frequently endows them with the capacity to stimulate humoral and cellular immune responses in the species from which the antigen originated. Drug-induced autoimmune responses are well documented; for example, autoimmune hemolytic anemia develops in association with the administration of methyldopa, and production of antinuclear antibodies follows treatment with hydralazine, isoniazid, or procainamide. Parenthetically, a genetically controlled polymorphism of the hepatic acetyltransferase enzymes is responsible for different rates of inactivation of these drugs. Slow acetylators are more prone to develop antinuclear antibodies with hydralazine ingestion, but there is no predominance of slow acetylators among SLE patients, nor are slow acetylators at greater risk of development of the disease. High titers of cold agglutinins to the I blood group arise as an occasional complication of *Mycoplasma pneumoniae* infections. The autoantibody persists for only a few days but is associated with a short-lived and sometimes severe hemolytic episode. The cold agglutinin is thought to be a cross-reacting autoantibody arising from the response to I-like determinants of the *Mycoplasma*. Anti-I cold agglutinins also develop in rabbits injected with group C streptococcal vaccine, owing to cross-reactivity of the I blood group substance with the immunodominant sugar moiety of the group C carbohydrate. A similar situation probably occurs in rheumatic fever of humans, in which certain streptococci carry antigenic determinants that cross-react with heart muscle or neuronal tissues, resulting in Sydenham's chorea. The brain and nerve damage sometimes occurring in humans after rabies vaccination may develop in much the same way if the rabies vaccine is prepared from heterologous brain tissue, and the same is true of autoantibodies evolving in patients receiving animal hormone replacement therapy, eg, bovine insulin or ACTH. There is also evidence that antigens common to *Trypanosoma cruzi* and cardiac muscle provide some of the immunopathologic lesions seen in Chagas' disease. Additional examples of possible involvement of antigenic mimicry in the pathogenesis of certain autoimmune diseases are (1) the cross-reaction of antibodies to measles virus with myelin basic protein, an antigen considered of importance in multi-

ple sclerosis; (2) the cross-reaction of anti-acetylcholine receptor antibodies with bacterial *(Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae)* extracts; (3) the induction of anti-DNA autoantibodies with cardiolipin and, conversely, the reactivity of anti-DNA autoantibodies with a variety of phospholipids and bacteria; (4) the idiotypic and structural similarities between some anti-DNA autoantibodies and certain anti-*Klebsiella* paraproteins; (5) the presence in synovial membranes in rheumatoid arthritis of a protein that shares an antigenic epitope with the Epstein-Barr virus (EBV)-encoded nuclear antigen; and (6) the structural similarities between α-gliadin, a wheat protein known to activate celiac disease, and a protein of adenovirus type 12.

Thymic Defects

The thymus, via its epithelial microenvironment and its giant nursing cells, as well as hormones such as thymopoietin and thymosin, is essential for the differentiation of T cells and their helper, suppressor, and cytotoxic subsets. It is unclear whether generalized autoimmunity such as that in SLE can result from intrinsic thymic abnormalities, although a variety of related pathologic and hormonal thymic abnormalities have been described.

All SLE mice develop early thymic atrophy, particularly involving the cortex and, to a lesser extent, the medulla. Such thymic atrophy associated with abnormal fine structure appears by the fourth month in (NZB × NZW)F_1 mice, which at 6–7 months of age have lost 70–90% of their cortices. In BXSB and MRL/l mice, which die earlier than the (NZB × NZW)F_1 mice, thymic atrophy and cystic necrosis appear by 2 months of age and progress to complete loss of the cortical areas by 4½ and 3½ months of age, respectively. In addition, some have reported that adult NZB mice lack a serum activity, thought to be a thymic hormone, and that administration of thymic hormone (ie, extract of thymic tissue) to NZB mice or transplantation of thymuses or of thymocytes from young NZB mice to the older mice may temporarily prevent some of the immunologic defects and delay the onset of autoimmunity. However, others have failed to inhibit the disease in autoimmune mice treated repeatedly with thymocytes from young counterparts, and attempts to confirm the therapeutic efficacy of thymosin in NZ mice have also failed. Moreover, thymectomy in newborn (NZB × NZW)F_1 and BXSB mice has little effect on the time of onset, mortality rate, or development of SLE in these strains of mice, and congenitally athymic (NZB × NZW)F_1 mice develop disease like their euthymic counterparts. Furthermore, transplantation and exchange of thymuses between the congeneic MRL/l and MRL/n mice, which exhibit markedly differential expressions of SLE—the former die by the fifth month of age with SLE, whereas the latter have very late developing disease, with death occurring at around the second year of life—showed that the genotype of the thymus is not the determining factor in expression of this disease. MRL/l mice thymectomized at birth and then transplanted with either MRL/l or MRL/n thymus developed equally early disease, and MRL/n mice thymectomized and transplanted with MRL/n or MRL/l thymus developed late disease like the nonthymectomized counterparts. However, in contrast to the situation in NZ and BXSB mice, MRL/l mice expressed the disease only in the presence of a thymus, irrespective of its genotype, and neonatally thymectomized animals did not develop SLE-like disease nor this strain's characteristic massive T cell proliferation. Although these experiments strongly suggest that thymuses of autoimmune mice are not intrinsically abnormal, they do not exclude the possibility that secondary thymic defects or accelerated thymic involution occurring in such mice or humans via a variety of means, including thymocytotoxic antibodies, may accelerate the autoimmune manifestations.

Current and future molecular analyses of the ontogenic development and structural integrity of T cell receptors for antigen may provide further insight into the role of the thymus and T cells in autoimmune pathogenesis. Recent studies by restriction fragment length analysis of genomic DNAs of lupus mice indicate that the C_α and C_β genes of these mice are structurally normal with the exception of NZW mice, wherein a deletion of the $C_{\beta 1}$-$D_{\beta 2}$-$J_{\beta 2}$ complex was identified. The contribution of this deletion to disease pathogenesis in NZ mice remains to be determined. Furthermore, similar detailed studies in MRL/l mice indicate that their proliferating cells express T cell receptor protein on their surfaces but at a reduced density compared to normal mice. However, normal levels of T cell receptor α, β, and γ mRNA were found in these cells as compared to normal lymph node cells. Southern blot analysis of MRL/l lymph node DNA showed rearrangement in 80–90% of the chromosomes at the β gene loci, and the pattern of rearrangements indicated that the T cell expansion in these mice is not monoclonal in nature. However, analysis of a number of cDNA clones from MRL/l lymph node libraries has suggested that the proliferating T cells, although utilizing a variety of J_β elements and both D_β elements (thus being idiotypically polyclonal), exhibit a restricted heterogeneity in their V_β gene expression, with the $V_{\beta 8}$ subfamily being the most abundant. This finding has certain implications with regard to the state of maturation of these cells and the mode by which they accelerate autoimmune disease.

Exogenous Polyclonal B Cell Activators

The cardinal feature of many—if not all—autoimmune diseases is the production of antibodies against numerous self antigens by the B cells. Although such an abnormality may be secondary to T cell defects (enhanced helper or reduced suppressor function), the alternative possibility that one or more defects at the B cell level are the primary causes of autoimmunity must also be considered. Of course, such B cell defects might secondarily induce T cell abnormalities that could accelerate the disease process. The

B cell defects might be intrinsic and genetically imposed—eg, an ability of certain clones of autoreactive B cells to hyperrespond to various stimuli—or might be extrinsic—eg, activation of B cells by endogenous or exogenous mitogens, the so-called polyclonal activators.

The proposition that polyclonal B cell activators can induce autoantibody production is based on the existence in the body of B cells that are not tolerant to self and the ability of B cell mitogens to stimulate these cells directly or by substituting for helper T cells. Thus, when self antigens are present in low concentrations, B cells with receptor reactivities ranging from low to high avidity are believed to escape tolerance induction and assume competence to interact with autologous antigens while T cells are rendered tolerant. Polyclonal B cell activators may trigger such B cells to produce autoantibody.

A variety of bacterial products, some viruses or viral components, parasites, and other substances may act as polyclonal B cell activators (Table 11–4). The fact that so many exogenous substances can act as polyclonal B cell activators has led to great interest in their ability to induce antibodies directed against the body's own components. Considerable data suggest that this occurs, as indicated by the development of rheumatoid factors and antinuclear, antilymphocyte, antierythrocyte, and anti-smooth muscle antibodies after bacterial, parasitic, or viral infections. Moreover, bacterial lipopolysaccharide induces murine lymphocytes to form a variety of autoantibodies, predominantly IgM class, such as anti-DNA, anti-γ-globulin, antithymocyte, and antierythrocyte autoantibodies. Injection of mice with lipopolysaccharide induces IgM responses against self IgG that account for a considerable percentage of the total number of IgM-producing cells. Many other bacterial products also provoke polyclonal B cell activation, but their ability to induce autoantibodies remains unknown.

Table 11–4. Polyclonal activators of B lymphocytes.*

Lipopolysaccharide (LPS)
PPD
Staphylococcus aureus protein A
Nocardia water-soluble mitogen
Lipid A - associated protein
2-Mercaptoethanol (2-ME)
α-Thioglycerol (α-TG)
Macrophage- and T cell - derived lymphokines
Fc fragment of immunoglobulins
Proteolytic enzymes, eg, trypsin
Polyanions, eg, dextran sulfate, poly I-C
Antibiotics, eg, nystatin, amphotericin B
Lanatoside C
Mycoplasma
Some viruses and viral components (EBV, gp70, measles)
Parasites *(Trypanosoma brucei, Trypanosoma cruzi, Plasmodium malariae)*

*Reproduced, with permission, from Goodman MG, Weigle WO: Role of polyclonal B-cell activation in self/non-self discrimination. *Immunol Today* 1981;2:54.

Protozoan parasites such as *Trypanosoma brucei, Trypanosoma cruzi,* and *Plasmodium malariae* are also polyclonal B cell activators, and autoimmune responses to DNA, red blood cells, and thymocyte antigens have been observed in animals experimentally infected with *T brucei*. Finally, some viruses act as polyclonal B cell activators, the best-known of which is EBV.* Peripheral mononuclear cells from normal persons and patients with rheumatoid arthritis become activated to secrete polyclonal IgG and IgM anti-γ-globulin antibodies during incubation with EBV, but cells from patients with rheumatoid arthritis produce much greater quantities of antibody with higher affinity than cells from normal persons. EBV activates the B cells directly, without requiring the participation of helper T cells, although T cells can suppress this process. Thus, during EBV-induced infectious mononucleosis, suppressor T cells become activated, preventing B cell activation in vitro by EBV as well as by other polyclonal B cell activators such as pokeweed mitogen. Cultured cells from patients with rheumatoid arthritis generate much less T cell-mediated suppression for EBV-induced polyclonal B cell activation than normal cells. This defect in suppressor T cell function is specific for EBV-induced polyclonal B cell activation, while other T cell suppressor functions such as Con A-induced suppression remain within normal limits. In addition, the defective T suppressor function described in patients with rheumatoid arthritis is not a common feature of many other autoimmune diseases, including SLE. The relationship of EBV to rheumatoid arthritis is discussed further in the section on viral factors in autoimmunity.

Many other factors such as Fc fragments of immunoglobulins, proteolytic enzymes such as trypsin, polyanions, and certain antibiotics may express properties of polyclonal B cell activators, but whether or not they can induce autoantibodies has not been determined. In general, autoantibodies induced by polyclonal activators are transient, of low affinity, and primarily of the IgM isotype, although induction of IgG-type rheumatoid factors and anti-DNA by certain forms of lipopolysaccharide, especially those high in lipid A, has recently been demonstrated. Moreover, in vivo prolonged polyclonal activation of B cells by the nonantigenic but potent mitogenic lipid A portion of lipopolysaccharide resulted in acceleration of the late-life SLE of female MRL/n, BXSB, and NZW mice, but it had relatively mild pathologic consequences in immunologically normal mice. These findings indicate that polyclonal activators per se cannot induce chronic disease unless there is a genetic predisposition. Whether—and, if so, how often—such polyclonal activation occurs in vivo is unclear, but it may be partly responsible for the low levels of autoreactive antibody found in the sera of normal individuals. However, these autoantibodies are usually of such low avidity that they are of little significance. Moreover, transfer of autoimmunity with bone marrow of SLE mice into histocompatible normal mice and the absence of autoimmunity after reciprocal transfers of

normal bone marrow cells into SLE strains further suggests the irrelevance of polyclonal non-lymphoid cell-associated activators as primary causative agents of murine SLE. This conclusion is reinforced by the following observations: (1) known potent polyclonal B cell activators induce primarily IgM autoantibodies, yet the tissue damage that accompanies most autoimmune diseases is mediated by IgG autoantibodies; (2) induction of autoantibodies by polyclonal activators is transient and disappears with elimination of the activator; and (3) no endogenous polyclonal B cell activator has been proved as an exclusive property of sera or tissue extracts of animals or humans with SLE. These arguments, of course, do not exclude a secondary role of polyclonal B cell activators as accelerators of autoimmunity in genetically predisposed individuals.

Macrophage Defects

There is general agreement that the mononuclear phagocyte serves an essential role in the cellular and molecular events that underlie immune competence by processing and presenting antigen to lymphocytes and by generating a number of humoral factors that influence the activities of lymphocytes. Moreover, the phagocytic function is important in disposal of immunologically undesirable materials such as immune complexes. Surprisingly, little information is available on the functional state of mononuclear phagocytic cells in autoimmune disorders. Most experiments deal with the capacity of macrophages from mice and humans with generalized autoimmune diseases to process and degrade antigens and the ability of the reticuloendothelial system to remove immune complexes or other particles from sera of such individuals. Most reports on the phagocytic activity of NZB-derived macrophages have shown elevated activity in terms of antigen phagocytosis, although some data suggest that the NZB cells do not effectively degrade the ingested antigen. Studies on the clearance of inert particles or immune complexes by macrophages of NZ mice are inconclusive. Some have shown a reduction in clearance; others portray clearance as increased or normal; and still others claim normal clearance but weak affinity for binding to Fc receptors of Kupffer's cells and therefore easy re-release into the circulation. Decreased in vivo clearance of antibody-sensitized red blood cells has been described in humans with autoimmune disorders. Whether this is a primary defect resulting from reduced Fc receptor number or abnormal function or secondary to occupation of the receptors by circulating immune complexes formed in vivo is not clear at present. However, recent studies indicate that the numbers of IgG Fc receptors on mononuclear cells of SLE patients are normal or increased, and there is no correlation between immune complex levels and degree of the clearance defect. Such IgG Fc receptor defects have also been observed in certain normal individuals and found to be associated with HLA-B8/DR3, HLA-DR2, or MT1 haplotypes. In addition to Fc receptors, defects in the number of CR1 (C3b, immune adherence) receptors present on surfaces of red blood cells from patients with autoimmune diseases such as lupus and rheumatoid arthritis have been revealed recently. As in the case of Fc receptor defects, it is not yet known whether this defect is genetic or secondary to the disease. However, whatever the cause, such reduced numbers of receptors related to phagocytosis and modulation of immune complex-bound C3b (CR1 receptors play primary roles in the degradation of C3b to smaller fragments) could lead to a decreased clearance of immune complexes and a greater deposition in tissues such as the kidney.

Phagocytes are essential for the development of various lymphocyte functions, particularly those of the T lymphocytes. As stated above, most T lymphocyte activities require that the macrophage take up and present the antigen in a process modulated by the I region of the MHC. Macrophages from newborn mice, tested at an age when immune responsiveness is low and tolerance is easily induced, present antigen poorly. This defect has been correlated with the small number of macrophages that bear Ia antigens in spleens of neonatal mice compared to adult mice. Whether the ontogenic development of Ia antigens on macrophages of autoimmune and normal mice differs (earlier development in the former than the latter), accounting for the lack of tolerance to self, remains to be determined. Recent studies have shown that ontogeny of Ia^+ macrophages early in life is normal in all lupus strains, but as the mice age there is an increased frequency of Ia^+ peritoneal macrophages in MRL/l and NZB mice. The significance of this finding is not clear at present, although a hypothesis has been advanced that overproduction of certain lymphokines, such as interferon, and increased T cell response to Ia, as well as enhanced Ia expression, may be responsible for the MRL/l lymphoproliferation. However, this abnormality is neither a prerequisite nor a general characteristic of murine SLE, since NZB/W and BXSB mice do not exhibit this defect. Furthermore, other *lpr* gene homozygous mice (C57BL/6-*lpr*, C3H-*lpr*) develop lymphoproliferation and autoantibodies without increases in the frequency of Ia^+ macrophages.

Aberrant Expression of Class II MHC Antigens

The first step in the induction of humoral or cell-mediated immune responses requires the interaction between antigen-presenting cells and T lymphocytes restricted at the major histocompatibility complex (MHC). Antigen-presenting cells (macrophages, B cells) invariably express MHC class II molecules (HLA-DR in humans and Ia in the mouse) that are recognized by T cells of the helper/inducer subset in association with antigen fragments. The involvement of qualitative or quantitative changes in DR/Ia molecules has been suggested in the pathogenesis of systemic and organ-specific autoimmune diseases. Thus, Gleichmann and associates have emphasized the unique potential of altered MHC determinants and provided experimental evidence in model systems that recognition of foreign or altered Ia antigens by T cells can lead

to an SLE-like illness. For example, if the F_1 hybrids of congenic mouse strains which differ at an Ia locus (I-E) are given parental cells from one of these strains, the recognition by the infused parental T cells of the Ia antigens of the other parent results in a graft-versus-host reaction that induces hypergammaglobulinemia and a variety of autoantibodies characteristic of SLE. Gleichmann postulated that lupus may result from a combination of class II self-MHC with foreign antigens such as drugs and infectious agents. This new site is then recognized and reacted against by helper T cells essentially in the same way as the allogeneic MHC structures on F_1 cells are recognized and reacted against by parental T cells. The stimulated T cells then release various lymphokines that induce terminal differentiation of activated self-reactive B cells in an antigen-nonspecific fashion. B cell clones recognizing cell-membrane epitopes and antigens with repeating epitopes such as DNA and phospholipids are preferentially engaged in this process, since such cells—in contrast to those recognizing globular proteins—are more likely to be activated and express acceptor sites for the antigen-nonspecific lymphokines. In this way, although the lymphokines driving B cells into terminal differentiation are antigen-nonspecific, the response is not a random polyclonal event but a highly selective process. This concept was later extended and modified by Eisenberg and Cohen, who proposed—in contrast to the Gleichmann model—that the T cells engaged in this process are autoantigen-specific. According to these investigators, T cells tolerant to self antigens are able to respond once these antigens are presented together with altered Ia antigens. These 2 possible mechanisms are not mutually exclusive, since both specific and nonspecific abnormal T cell help could be simultaneously induced by altered perception of Ia.

Genetic or induced variations in levels of autologous (unmodified) DR/Ia molecules have also been implicated in the pathogenesis of autoimmune diseases. Increased DR/Ia expression by antigen-presenting cells may stimulate strong autologous mixed lymphocyte reactions via which T cell-derived lymphokines can induce differentiation of autoantigen-activated B cells. Alternatively, as proposed by Bottazzo and associates, class I or class II MHC antigens may be ectopically expressed, thereby allowing helper/inducer or cytotoxic T cells, respectively, to accumulate in these sites. By means of immunofluorescence and a monoclonal anti-DR antibody, these investigators found that the normal thyroid epithelium does not express DR antigens. In contrast, high epithelial cell staining was demonstrated in most thyroids from patients with Graves' disease. These findings may explain how T lymphocytes recognize thyroid autoantigens. The aberrant production of DR antigen exposes the MHC class II structures that helper lymphocytes require for antigen recognition. The thyroid epithelial cell then becomes an antigen-presenting cell. The mechanisms responsible for the aberrant expression of DR antigens are not known. Evidence has been obtained that interferon induces epithelial cells (including thyroid epithelium) to express such antigens. Therefore, it has been hypothesized that a local viral infection, by eliciting the production of interferon, could activate DR expression and thus permit recognition of thyroid autoantigens by T cells. Similar mechanisms have been implicated in the pathogenesis of other organ-specific autoimmune disease, particularly autoimmune endocrinopathies and diseases of the central nervous system such as multiple sclerosis.

Defects In Tolerance Induction

Studies have been performed in mice with SLE to determine whether there are defects in tolerance induction. Indeed, all murine SLE strains studied were defective in tolerance induced to deaggregated human gamma globulin, bovine gamma globulin, or hapten-substituted gamma-globulin in vitro and in vivo. Some studies attributed this defect to T cells, others to B cells, and yet others to both levels. Whether difficulty in tolerance induction applies to antigens other than deaggregated gamma globulins, both exogenous and endogenous, has not been investigated comprehensively. However, experiments with hapten-modified self cells have shown that MRL/l mice are as easily tolerized as immunologically normal mice, suggesting that the defect might not be universally applicable to all antigens. Moreover, studies in F_2 generations of SLE mice have failed to show any correlation between tolerance inducibility and disease expression.

Defects of Multipotential Stem Cells

At present, the best way of defining the humoral, cellular, microenvironmental, and viral factors from which autoimmunity develops is to transfer autoimmune disease by using specific tissues or tissue extracts from strains of animals having a genetic predisposition to autoimmunity into recipients without this defect. Such transfer studies have been performed among SLE murine strains and their normal counterparts. Thus, NZB autoimmune disease has been transferred with this strain's fetal liver, bone marrow, or spleen cells into lethally irradiated, normal histocompatible strains of mice. Lethally irradiated NZB mice transplanted with H-2-compatible allogeneic normal mouse bone marrow or spleen cells do not develop autoimmunity. In some reverse transfers, NZB bone marrow cell suspensions were depleted of T cells, the H-2-compatible normal recipients were thymectomized, or anti-T cell antibodies were given after transplantation and disease developed nonetheless. These experiments have been cited as evidence that many of the NZB peculiarities may be intrinsic to hematopoietic cells. Similar experiments have been performed between BXSB male and female SLE mice. The male BXSB mice develop severe early SLE with 50% mortality rates at 5–6 months of age, whereas the females develop late SLE with 50% mortality rates after 18 months (Table 11–3). Reciprocal transfers of bone marrow or spleen cells between these mice show that male cells can transfer early disease in both le-

thally irradiated male and female recipients, whereas female cells cause late disease regardless of the recipient's sex. Further experiments indicate that transfers of spleen cells from older male BXSB mice with clear-cut disease produce disease in recipients no faster than transfer of cells derived from premorbid mice. The conclusion is, therefore, that the active cells in these transfers are stem or precursor cells—not autoantibody-secreting B cells—and that the development of BXSB disease does not result from an accumulation of defects among stem cells, which are equally abnormal throughout the animal's life. Like the NZB mice, transfer of BXSB male disease by bone marrow cells can be accomplished in the absence of T cells in the inoculum and in thymectomized female recipients. This finding implies that the defect of this strain of mice is associated with precursors of the B cell lineage. However, as discussed below, the primary B cell defect of NZ and BXSB mice needs accessory cell (T cell, macrophages)-derived signals for expression.

Defects in Production of & Response to Accessory Signals for B Cell Proliferation & Differentiation

The most consistent characteristic underlying the diverse clinical features of SLE in humans and murine experimental models is a substantial increase in the number of spontaneously activated B cells and polyclonal immunoglobulin-secreting cells (IgSC). Until recently, little was known about the cause of this B cell hyperactivity, and T cell defects were frequently invoked as a possible explanation. However, as stated above, no generalized suppressor T cell defect has been confirmed in either human or murine SLE.

Over the past few years it has been shown that lymphocyte activation, proliferation, and differentiation proceed in a stepwise manner and involve not only antigen presentation and recognition but also a cascade of antigen-specific and -nonspecific amplification signals mediated by soluble factors (Fig 11–7). Accordingly, resting T cells evidently require at least 3 signals to perform their effector functions. Signal 1—antigen binding—renders the selected T cells sensitive to the inductive signals 2 and 3—the interleukins, namely, interleukin-1 (IL-1), which is the product of macrophages, and interleukin-2 (IL-2), which is the product of activated T cells. IL-1 and IL-2 provide the additional signals necessary for proliferation and differentiation of the antigen-selected T cells to effector cells.

Although incomplete, recent studies also indicate that a resting B cell, like the T cell, becomes transformed into an expanded population of antibody-secreting cells only after a series of proliferative and differentiating events occur, and these appear to be regulated by separate soluble factors and signals. Generally, resting B cells apparently need at least 3 different signals to generate clones of antibody-producing cells in either a T cell-dependent (TD) or so-called T-independent (TI) B cell response. For TD responses, one signal (the antigen-specific T_H signal) is generated when T_H cells see Ia determinants together with antigen (carrier) on the B cell; another is generated when B cells specifically see antigen (hapten) via surface immunoglobulin. These 2 signals are required to activate resting B cells from the G_0 I G_1 phase of the cell cycle, at which time the activated B cells acquire receptors or acceptor sites for a third signal that is mediated by Ia- and antigen-nonspecific T_H cell-derived factors. In contrast to the TD antigens, for which all 3 signals are required, for TI antigens the requirement of the MHC-restricted T_H signal can be bypassed either by the polyclonal B cell activity of TI-class 1 antigens (LPS) or by cross-linking of the B cells' antigen receptors by the multivalent TI-class 2 (Ficoll) antigens. Despite bypassing of the MHC-restricted step, antigen-nonspecific T_H-derived factors are still necessary for responses to TI antigens. Therefore, antigen-nonspecific T_H-derived signals are required as a final common pathway in the activation of B cells by both TD and TI antigens.

Studies with products of nonhomogeneous T cell populations stimulated with mitogens, T cell hybridomas, and antigen-specific T cell clones suggest that the T_H factors acting on B cells can be classified into 2 general categories: B cell growth factors (BCGF),

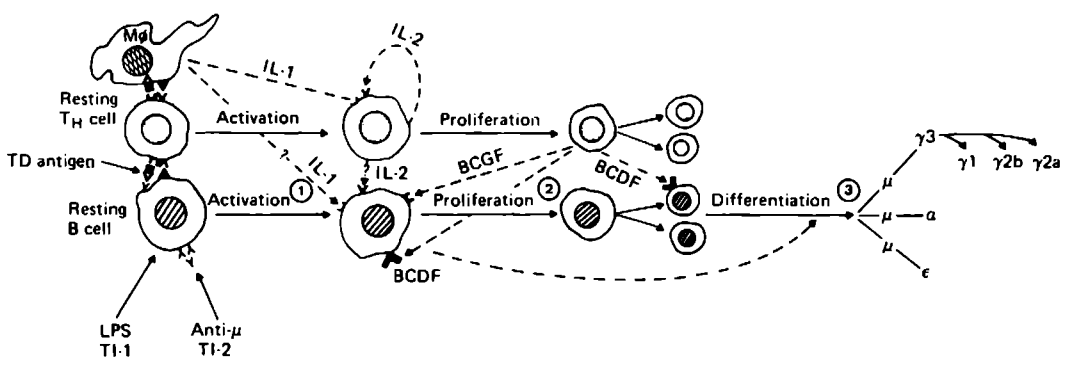

Figure 11–7. Signal requirements for B cell and helper T cell (T_H) activation, proliferation, and differentiation (see text).

which stimulate proliferation; and B cell differentiation factors (BCDF), which stimulate maturation and immunoglobulin secretion. Proliferation and differentiation appear to be independently controlled events. Some investigators have found that IL-1, IL-2, interferon, and another factor (T cell replacing factor [TRF]) may also act directly on B cells. The T_H cell-derived differentiation factors have been found to be capable of inducing immunoglobulin isotype switching and to have isotype specificity. Thus, differentiation factors specific for IgM, IgA, IgG, and probably IgE have been described. As for the IgG subclasses, IgG1 and IgG2 are influenced more by T cell products than IgG3, for which no T cell influences have been reported. A T cell influence on immunoglobulin switching has been shown not only for TD antigens but also for TI-2 and perhaps TI-1 antigens.

From the above description of signals and factors that influence the amount and quality of an antibody response, it becomes apparent that defects in signal requirements, overproduction of factors, or abnormal responses of B cells to such factors may cause hypergammaglobulinemia, autoantibody production, immunoglobulin switch into pathogenic subclasses of autoantibodies, and finally a generalized autoimmune disease such as lupus.

Recent studies, therefore, questioned whether the B cell responses of SLE mice differ from the normal responses outlined. The answer is clearly yes in some strains, but these differences are mainly quantitative rather than qualitative.

A variety of experiments led to the following conclusions:

(1) B cell proliferation and differentiation in SLE mice remain dependent on accessory signals of both macrophage and T cell origin. The requirement for T helper signals was demonstrated not only by in vitro studies but also by in vivo experiments, where it was shown that elimination of $L3T4^+$ helper cells with appropriate antibodies inhibited disease development in NZB/W mice.

(2) B cells from BXSB, NZB/W, and MRL/l mice appear to require the same number of signals as normal B cells to undergo polyclonal- or antigen-directed responses. B cells of BXSB and NZB/W (but not MRL/l) origin differ from normal B cells by the higher sensitivity (or degree of response) to the signals they receive.

(3) Proliferating T cells in enlarged nodes and spleens of older MRL/l mice, in the absence of mitogens, secrete in vitro abnormally high levels of a factor that induces terminal differentiation of activated B cells to immunoglobulin-secreting cells.

Based on the above data, murine SLE was subdivided into 2 types:

Type 1 SLE: The defining characteristic is a genetically determined primary B cell defect that results in excessive and poorly regulated polyclonal B cell activation, proliferation, and differentiation. This type of B cell hyperactivity is found in BXSB and NZB/W mice and may represent the most common type of human SLE. The signals that activate B cells in this disease may include exogenous and endogenous antigens and mitogens. Macrophage- or T cell-derived signals are still required to drive immunoglobulin secretion, but clearly in this case normal levels of help are sufficient to cause excessively high levels of immunoglobulin production.

Type 2 SLE: The definitive quality is that T cells chronically hypersecrete helper T factors, which either induce an abnormal number of B cells to secrete immunoglobulin or induce more immunoglobulin secretion per B cell. B cells appear to be normal. In mice, this form of SLE is found in strains carrying the lpr (lymphoproliferation) gene, eg, MRL/l mice. Here, basal immunoglobulin secretion is shifted to extremely high levels (especially IgG1 and IgG2) such that multiple autoantibodies, which are normally found only in very low titers, are secreted at levels sufficient to cause pathogenic immune complexes.

Based on these findings, it could be postulated that a systemic autoimmune disease such as lupus is caused by increased polyspecific immunoglobulin and autoantibodies above a subclinical threshold level. Normally, this threshold level, constituting the so-called basal immunoglobulin secretion, is under homeostatic control until late in life, when the balance may tilt in favor of help rather than suppression. In early-life lupus, this background immunoglobulin is greatly increased, because the system is constantly hyperstimulated either by hyperresponsiveness to or hyperproduction of accessory signals. Viewed in this way, lupus results from a disturbance of the homeostatic mechanisms that normally control background or basal immunoglobulin secretion. The predominance of certain autoantibodies over others may be related to the amount or special properties of certain autoantigens that would permit them to activate B cells, or to a genetic predisposition for high- and pathogenic-quality response to these antigens.

Interestingly, addition of the xid gene (X-linked immunodeficiency gene) onto the genome of mice with either type 1 or type 2 SLE considerably lessens the severity of disease. The xid gene results in deletion of the Lyb 5^+ B cell subset. This subset is required for responses to some types of TI antigens (TI-2 antigens), represents a more mature subset of B cells, and appears to act as the target for antigen-nonspecific T_H-derived signals.

Other Lymphokine Defects

Lymphokines or interleukins are a family of molecules transmitting growth and differentiation signals between various types of leukocytes and thus presumably are major effectors of immune regulation, as discussed above.

Among the various interleukins, interleukin-2 (IL-2) is believed to provide a universal signal for proliferation and differentiation of antigen-activated T cells (and B cells?) through its binding to specific cell surface receptors. Because of the central role believed to be played by IL-2 in regulating T cell responses and in

view of the immunoregulatory abnormalities of SLE-prone mice, several investigators have performed detailed analyses of IL-2 production and consumption by T cells of autoimmune mice and their relationship to the disease process.

Studies by Altman and associates demonstrated that age-dependent reduced Con A-induced mitogenic response and IL-2 production are a common feature of all SLE strains. This defect appears at 3–6 weeks of age in the early-life, severe SLE-developing MRL/l and male BXSB strains and progresses thereafter. Similar defects appear at a later stage in NZ, MRL/n, and female BXSB mice. Detailed analysis of cells from the enlarged lymph nodes and spleens of older MRL/l mice demonstrated that such cells (1) respond poorly to Con A or allogeneic stimulator cells, even in the presence of exogenous IL-2; (2) did not suppress IL-2 production by normal spleen cells; and (3) were relatively incapable of adsorbing or inactivating exogenously added IL-2. These results indicate that T cells of MRL/l mice are severely defective in their responses to mitogenic stimuli, IL-2 production, and IL-2 receptor site expression. Similar results were reported by Talal and associates and extended to other lpr homozygous mice such as the C57BL/6J-lpr/lpr. These investigators also found that the defect in Con A-induced IL-2 production could not be corrected by the presence of interleukin-1 (IL-1), a product of macrophages. It is notable that in all of the above studies, the IL-2 decrease could be detected prior to the massive T cell proliferation of lpr homozygous mice, suggesting that this abnormality is not caused by dilution of normal IL-2-producing cells by the proliferating T cells.

At any rate, the causes of decreased IL-2 production and response in lupus mice cannot be determined at present, but they do not appear to involve increased suppressor T cell function or inefficient IL-1 production. Impaired cell maturation or occupation of IL-2 receptors by passive in vivo absorption of IL-2 has not been excluded. The relationship of the IL-2 defect to the disease process also remains unclear. It should be noted that several studies in humans with SLE or rheumatoid arthritis also found defective IL-2 production, and one study showed defective IL-1 production. The availability of cloned human IL-2, as well as the recent development of monoclonal antibodies to conventional IL-2, recombinant IL-2, and chemically synthesized IL-2, may allow the precise definition in vitro and in vivo of the defects observed and of the potential usefulness of IL-2 in manipulating systemic autoimmune and other diseases.

Apart from the IL-2 defects, qualitative and quantitative abnormalities in interferon have been described in systemic autoimmune diseases. Interferons (IFN) are classified into 3 groups on the basis of their antigenic properties: (1) IFN α, produced mainly by leukocytes in response to viral and nonviral stimuli and stable at pH 2; (2) IFN β, synthesized predominantly by fibroblastlike cells and, to a much lesser extent, by leukocytes and also acid-stable; (3) IFN γ, or "immune IFN," released by lymphocytes following exposure to mitogens or specific antigens (see Chapter 8). IFN γ is inactivated by incubation at pH 2 and is generally more heat-labile than IFN α or β. Both IFN α and IFN γ may be involved in the regulation of immune responses in vivo. Furthermore, IFN γ in mice has recently been shown to be identical to macrophage activating factor (MAF) and perhaps to macrophage Ia inducing/recruiting factor (MIRF). Mouse IL-2 (derived from Lyt 1^+ T cells) appears to be involved in the induction of IFN γ by predominantly Lyt 2^+ T cells.

Initial studies demonstrated high levels of undefined species of interferon in SLE patients, particularly those with active disease. These findings were subsequently confirmed in part, but no correlation with clinical activity was observed. Furthermore, it was found that this was an IFN α, although it was acid-labile like the immune IFN γ. An increase in interferon-induced 2′,5′adenylate synthetase in mononuclear cells of SLE patients was also identified.

With regard to murine SLE, normal levels of IFN γ in Con A-stimulated MRL/l lymphocytes were reported despite the reduced IL-2 levels in similar cultures. However, subsequent studies did not confirm these findings but demonstrated that Con A-stimulated lymphocytes from all early-life lupus substrains produced lower levels of IFN γ than normal controls. The significance of these findings remains unknown. It should be noted, however, that NZB/W mice treated with IFN γ inducers or IFN γ itself showed accelerated disease manifested by earlier death and glomerulonephritis.

Defects in Idiotype-Anti-idiotype Network & Idiotype Mimicry of Autoantigen

As stated above, B and T cells and their soluble products may express idiotypic or anti-idiotypic determinants through which regulation of immune responses occurs. Anti-idiotypic antibodies may suppress or enhance immune responses. In most instances of autoanti-idiotypic responses to antibodies against exogenous antigens, the autoanti-idiotypic antibodies suppress the original immune response expressing the corresponding idiotype. In addition to anti-idiotype-mediated suppression, stimulation of Ab_1 by anti-idiotypes representing the "mirror image" of antigens that Jerne postulated has been described in recent experiments. Initial studies provided evidence that expression of idiotypes specific for Ab_1 may reappear on Ab_3, since Ab_4 bound to Ab_1 and Ab_1 itself inhibited the binding of radiolabeled Ab_3 to Ab_4. These results suggest that the immune idiotypic network is not open-ended but somehow circular. In further experiments, animals immunized with Ab_2 (anti-idiotype) developed not only Ab_3 (anti-anti-idiotype) but Ab_1 (idiotype) as well, suggesting that Ab_2 contains antigenic determinants (epitopes) conformationally similar to those on the inciting antigen, thereby fulfilling the predicted hypothesis of an "internal mirror image." Together, these observations suggest that a given anti-

body—as well as an autoantibody—could be viewed as the product both of original stimulation by the antigen and of stimulation by Ab_2 (anti-idiotype) that can internally mimic the antigen. The induction of idiotypes in the absence of antigen has been demonstrated in several systems. For example, induction of anti-tobacco mosaic virus antibodies in mice injected with specific anti-idiotypic antibodies has been observed. In this instance, Ab_2 behaved like the antigen: It reacted with anti-tobacco mosaic virus antibodies and also promoted the synthesis of anti-tobacco mosaic virus antibodies in the total absence of tobacco mosaic virus. Thus, these antibodies may be considered the internal images of tobacco mosaic virus. Furthermore, injection of experimental mice with anti-idiotypic antibodies to the murine MHC ($H\text{-}2K^k$ specificity) induced anti-$H\text{-}2K^k$ antibodies in the absence of exposure to $H\text{-}2K^k$ antigen. More importantly, recent studies by Zanetti have shown that antibodies specific for the idiotype of an autoantibody to thyroglobulin can perturb the natural mechanism of internal homeostasis or tolerance and induce autoantibodies to thyroglobulin in naive mice and rats. Generation of helper and killer T cell subsets via anti-idiotypic antibodies is also known.

In some instances, anti-idiotypic antibodies not only induced the idiotype but also mimicked functional properties of the inciting antigen unrelated to the latter's capacity to stimulate the immune system. For example, anti-idiotypic antibodies prepared against bovine anti-insulin mimicked the action of insulin by interacting with insulin receptors on tissues and stimulating the physiologic action of insulin itself. In this instance, a portion of the anti-idiotype, presumably part of its combining site, apparently resembled the insulin site reactive with insulin receptors. Similarly, anti-idiotypic antibodies against antibodies to retinol-binding protein or to alprenolol (a β-adrenergic antagonist) competed with retinol-binding protein or dihydroalprenolol binding to specific receptors on intestinal epithelial cells or red blood cells, respectively.

From these observations, one can speculate that autoimmune responses may be the result of defects in immunoregulation that allow underproduction or overproduction of anti-idiotypic antibodies. Such defects would permit either unchecked production of autoantibodies or cyclic stimulation of Ab_1 (idiotype) in the absence of the inciting antigen, respectively. Ultimately, such defects must be connected to the B cell-helper T cell-suppressor T cell circuit and their idiotypes and anti-idiotypes.

It is noteworthy that induction of idiotypes by anti-idiotypic antibodies that carry the image of antigens has recently been explored as a means of vaccination, and experimental induction of protective immunity against parasites, bacteria, viruses, and tumor cells has been reported.

To summarize: A variety of T and B cell immunologic defects may lead to the expression of transient or permanent autoimmune manifestations. No one of these mechanisms excludes the others; in fact, concerted action is likely, since defects of the T cell component are reflected by B cells and vice versa. Some of these abnormalities, such as in suppressor or helper cells, can be operative in either organ-specific or non-organ-specific autoimmune disease—whereas others, such as polyclonal B cell activators and early thymic involution, whose effects are non-organ-specific, would relate more closely to generalized diseases.

GENETIC FACTORS IN AUTOIMMUNE DISEASES

In human autoimmune diseases and in many induced or spontaneous animal models of autoimmunity, the role of genetic factors in determining the incidence, onset, and nature of the autoimmune process is clearly evident. However, in the case of most of these conditions, it has not been possible to attribute autoimmune predisposition to the action of a single genetic locus.

Primary candidates suspected of determining susceptibility or resistance to the development of autoimmune and, of course, other types of diseases are those genes that code for the magnitude and nature of immune responses to antigens. These are the MHC genes and the genes coding for antigen receptors on B and T cells. In regard to autoimmune diseases, associations with both MHC genes and immunoglobulin allotypic genes have been described. After reviewing the large number of studies available, one must conclude that most autoimmune diseases, at least in Caucasian populations, are associated, albeit not to a very satisfactory degree, with the alleles DR2, DR3, DR4, and DR5 (Table 11–5). Seropositive rheumatoid arthritis, which is independent of HLA-ABC, correlates chiefly with DR4, whereas SLE, once linked with HLA-B8, is now generally associated with DR2 and DR3. This distinction could imply a very different pathogenetic mechanism for these 2 diseases, usually considered to be closely related. Associations found for Caucasian populations may not apply for other ethnic groups, and vice versa. For example, Graves' disease is closely related to B8/DR3 in Caucasoids but to DR5 and DR8 in Japanese populations. Moreover, no uniform associations are observed among patients classified as having "organ-specific autoimmune diseases" or among those having "generalized autoimmune diseases," suggesting that each autoimmune disease has, at least in part, a distinct genetic background, although one frequently sees simultaneous expression of multiple organ-specific autoimmune diseases in a given individual and overlap of serologic findings. For example, there is a high incidence of pernicious anemia in patients with Hashimoto's disease (and vice versa); of Addison's disease in persons with autoimmune thyroid disease; and of rheumatoid factor and arthritis in patients with SLE. Furthermore, patients with organ-specific disorders are slightly more prone to develop cancer in the affected organ, whereas generalized lymphoreticular neoplasia shows up regularly along

Table 11–5. HLA-DR and diseases.*

	Antigen	Relative Risk
Multiple sclerosis	DR2	4.1
Optic neuritis	DR2	2.4
Goodpasture's syndrome	DR2	15
C2 deficiency	DR2	Linked to A25, B18
Dermatitis herpetiformis	DR3	15.4
Celiac disease	DR3	10.8 (also DR7)
Sicca syndrome	DR3	9.7
Addison's disease	DR3	6.3
Graves' disease	DR3	3
Juvenile diabetes	DR3	5.6 (also DR4)
Myasthenia gravis	DR3	2.5
Systemic lupus erythematosus	DR3	5.8
Idiopathic membranous nephropathy	DR3	12
Rheumatoid arthritis	DR4	4.2
Pemphigus	DR4	14.4
IgA nephropathy	DR4	4
Hydralazine-induced systemic lupus erythematosus	DR4	5.6
Hashimoto's disease	DR5	3.2
Pernicious anemia	DR5	5.4
Juvenile rheumatoid arthritis	DR5	5.2

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with non-organ-specific disease. The genetic, environmental, and immunologic factors predisposing to these combined abnormalities are not known. A high frequency of generalized autoimmune disease also accompanies deficiencies in certain complement components. Thus, patients with C2 deficiency, an autosomal recessive trait, often have vasculitis, skin rashes, recurrent infections, and a general picture similar to that of SLE. Similarly, a high incidence of SLE or similar syndromes characterizes patients with C4, C1r, C1s, or C3 deficiencies. Many autoimmune diseases also affect more females, suggesting a linkage with the X chromosome, although this association may be hormonally related (see below).

In general, typing for MHC specificities, particularly those encoded by the putative immune response class II locus of HLA, should eventually provide diagnostic benefits relative to clinical autoimmune disorders. These benefits include better definition of homogeneous subgroups of patients with a given disease, a more accurate prognosis for such patients, and identification of individuals likely to develop the disease—an indication of expected severity and development of preventive measures via genetic counseling. Future studies by molecular cloning techniques may allow a better understanding and more accurate definition of the associations of certain HLA-DR haplotypes with disease susceptibility. It is known, for example, that class II antigens show a marked genetic polymorphism associated with the β chain. Hybridization studies have shown that this chain is encoded by several genes. Hybridization patterns between DNA from healthy individuals and that from patients with insulin-dependent diabetes mellitus (IDDM) were recently analyzed using a β-chain cDNA probe and restriction endonucleases. Among HLA-DR4 and HLA-DR3/4 individuals, the IDDM patients showed an increased frequency of an 18-kb fragment. Moreover, a 3.7-kb fragment among controls (30–40%) was rarely detected in the IDDM patients (0–2%). Similarly, examination of the histocompatibility region of the nonobese diabetic (NOD) mouse indicated that this mouse has a unique class II MHC with no expression of surface IgE, no messenger RNA for IgE_a, and an IgA not recognized by appropriate monoclonal antibodies or T cell clones. In crosses of NOD mice with control C3H mice, the development of diabetes was dependent on homozygosity for the NOD mouse's unique major histocompatibility region. Involvement of MHC genes has also been implicated in the development of diabetes in the genetically susceptible BB rat. How MHC genes might be involved in the susceptibility to disease is not yet clear, but possibilities include effects on the magnitude of humoral and cellular responses and on immunoregulation (helper, suppressor, and cytotoxic T cell functions), metabolic influences especially on steroid hormones, effects on antigen handling by phagocytic cells, and antigenic mimicry. The possibility also exists that some of the associations between HLA and disease simply represent strong linkage disequilibrium (failure of adjacent genes to segregate in the population) between susceptibility genes and certain HLA haplotypes. Furthermore, some of the MHC-linked diseases at least may involve the interaction of 2 or more susceptibility genes, only one of which would be an MHC gene.

Certain genes coding for V regions (antigen-binding site) or C regions (effector function such as complement fixation and binding to cellular Fc receptors) of immunoglobulins also have an apparent association with particular diseases, including autoimmune diseases, as the importance of immunoglobulin allotypic

markers in determining susceptibility to autoimmune disorders suggests. For example, expression of rheumatoid factors in crosses of 129 and C57BL/6 mice depends in part upon a gene linked to the C locus. Thus, high levels of IgA anti-IgG2a (Igh-1) autoantibodies, like those found in sera of the 129 strain, appear only in Igh-1^{aa} mice, whereas IgM anti-IgG1 of the C57BL/6 type are detectable mainly in Igh-1^{bb} mice, and both types of rheumatoid factors are depressed in heterozygous Igh-1^{ab} animals. In other experimental murine systems, autoimmune manifestations and allotypes have been linked with antibody production against autologous erythrocytes, thyroglobulin, and acetylcholine receptor. Similarly, in humans, Gm allotypic homozygosity has been related to the risk of anti-γ-globulin development, and the presence of certain Gm allotypes was associated with autoimmune chronic active hepatitis, myasthenia gravis, SLE, insulin-dependent (juvenile-onset) diabetes with serum anti-insulin antibodies, and Graves' disease. Combined assessment of HLA haplotype and of Gm allotype in Japanese families that had more than 2 first-degree relatives affected by Graves' disease provided an excellent predictor of risk for this disease, since all affected individuals had a given combination of these 2 markers, although siblings who shared the disease-associated haplotypes did not necessarily suffer from the disease. Thus, one may expect that HLA and immunoglobulin allotyping, along with establishing other genetic markers and environmental factors, would allow fairly accurate prediction of autoimmune diseases in the future. In regard to allotypic immunoglobulin markers and diseases, it should be noted that idiotypic determinants coded by V genes have been linked to heavy chain C region allotypic markers of mice. Since idiotypic determinants on lymphocyte membranes appear to have fundamental roles in immune regulation, the development of techniques for their assessment, such as hybridoma technology that makes monoclonal idiotypes and anti-idiotypes available, may provide further means of improving genetic analysis of autoimmune disorders.

The genetic control and mechanisms of gene action in autoimmunity have also been studied in animals with spontaneous or induced autoimmune diseases. The best examples are mice with spontaneous lupus-like syndromes. The H-2 haplotypes, lymphocyte surface alloantigens, and IgG allotypes of this kind of mice are shown in Table 11–3. Unfortunately, no consistency or uniformity in any one of these markers is seen in these strains despite the expression of lupuslike syndromes in all of them. Two additional approaches have been used to determine the genetic factors associated with murine SLE: The first model results from crossing the several SLE-prone strains and then analyzing their F_1 hybrids in the hope of finding common genetic denominators among these strains; the second model involves analysis of F_2 hybrids and recombinant inbred strains so as to interrelate the individual immunopathologic and histocompatibility traits of SLE-prone strains. In initial studies by Adams and associates, offspring of (NZB × NZW)F_1 backcrossed with NZB and of (NZB × NZW)F_1 outcrossed with NZC mice were studied, and the genes determining expression of lupus nephritis and of autoimmune anemia, respectively, were assessed. Of the 3 genes necessary for the occurrence of lupus nephritis (lpn genes) in the NZB/NZW hybrid mice, one (lpn-1) is in the NZB strain and 2 (lpn-2, lpn-3) are in the NZW strain. Of the 2 genes governing autoimmune anemia (aia-1, aia-2) in the NZB/NZC hybrid mice, both are in the NZB strain and one (aia-2) is also in the NZC strain. Thus, the autoimmune diseases of these mice clearly depend on combinations of genes that are not pathogenic individually. Of these 5 genes, only one, lpn-2, is tightly linked to the MHC in these investigators' opinion. According to Adams, of the 3 genes governing lupus nephritis, none can be a heavy chain V gene, because these are on the murine chromosome 12 (in humans, on chromosome 14), whereas lpn-1 and lpn-2 are on chromosome 17, and lpn-3 is not linked to the heavy chain allotype, as clearly shown. Moreover, lpn-1 and lpn-2 are not kappa light chain V genes, since these are located on the murine chromosome 6 (in humans, on chromosome 2). The finding that aia-1, governing autoimune anemia, is on chromosome 4 again precludes its being a heavy chain or kappa light chain V gene. Similarly, these genes are not related to the murine lambda light chain gene or to the J chain gene, since the former is located on chromosome 16 (in humans, on chromosome 22) and the latter is located on chromosome 5 of the mouse. Therefore, the evidence indicates involvement in murine SLE of classes of genes additional to MHC and V genes. Studies by Raveche and associates similarly showed that antithymocyte production is controlled by a single codominant gene and that an independent dominant gene controls the production of autoantibodies to ssDNA. Apparently neither gene is H-2-linked. At present, the products of such codominant (ie, producing their effect in the heterozygous state), non-MHC, non-V genes are unknown. Additional studies in F_2 crosses of autoimmune mice performed by Dixon and associates, and in recombinant lines from crosses of NZB with normal C58 mice by Raveche, Steinberg, and Riblet and associates, suggest that neither of these 2 types of genes is involved in the pathogenesis of murine SLE. For example, it was found that the major controlling genes for expression of antierythrocyte and antilymphocyte antibodies are not structural genes for heavy or light antibody chains, since certain lines produced C58 heavy and light chains and yet were Coombs- or NTA-positive. Conversely, others had NZB heavy and light chains and did not produce anti-red cell or NTA autoantibodies. Furthermore, certain lines that had recombinant Igh haplotype with Igh-C genes from NZB and Igh-V genes from C58 still expressed high autoantibody levels. Finally, restriction fragment length analysis of genomic DNAs from lupus mice failed to disclose any gross abnormalities in their Ig-V_H loci corresponding to the seven V_H gene families. The results in toto suggest that the Ig-

V_H gene complex of lupus mice does not carry a primary defect responsible for autoimmune disease.

Further analysis of these SLE mice shows that individual accelerating factors characteristic for each strain account for differences in the onset and severity of disease as well as mortality rates among them (Table 11-3). In the BXSB mouse, the accelerating factor is associated with the Y chromosome but not with sex hormones and results in much earlier disease and death in males than in females. In the MRL mice, the accelerating factor is the autosomal recessive lpr gene that accounts not only for proliferation of Lyt 1^+ T cells but also for a significantly accelerated onset of disease in homozygous MRL/Mp-lpr/lpr mice compared to congenic MRL/Mp-+/+ mice that do not have the lpr gene. The significance of these genetically determined accelerating factors in the expression of murine SLE is indicated in F_1 hybrids of BXSB mice and in transfers of the lpr gene of MRL/l mice to other SLE and normal strains. When the BXSB mouse is used as mother, it complements the predisposition to lupus in both NZB and NZW strains and produces F_1 hybrids quite similar to the traditional (NZB × NZW)F_1 mice, with female offspring dying first; but BXSB females crossed with normal strains or MRL/l mice produce F_1s with little or no disease. However, when the BXSB is used as father in crosses with all other genetically predisposed SLE strains such as NZB, NZW, and even MRL/l, then a male-first early SLE develops. Similarly, establishment of the lpr gene in a homozygous state on NZB or MRL/n late-SLE-developing strains results in acceleration of the onset and course of lupus; for example, in NZB mice, the 50% mortality rate drops from 16 months to less than 5 months, and in MRL/n mice from 17 months to 5 months. However, in spite of inducing lymphoproliferation and autoantibodies, the lpr gene when introduced in normal background mice (C57BL/6, C3H) does not cause early SLE. In New Zealand hybrid mice, the female hormones (see below) apparently hasten disease onset and death in females compared to males. Thus, murine SLE is caused by many independently segregating genetic factors which in the presence of an endogenous or exogenous accelerator express themselves early, whereas in the absence of the accelerator they appear late in life. Future analysis of certain genetic markers whose location on a given chromosome is known and of their possible segregation or association with autoimmune phenomena and autoantibody production in appropriate recombinant and F_2 mice may pinpoint the exact location of the multiple abnormal genes responsible for this disease and provide the basis for further genetic characterization of humans with such multifactorial disease as SLE.

HORMONAL FACTORS IN AUTOIMMUNITY

Sex hormones as well as X chromosome- or Y chromosome-linked genes may influence the expression of autoimmune diseases. It is well known that hormones of the hypophysis, thyroid, parathyroid, adrenals, and gonads affect homeostasis of the lymphoid system and responses to antigens by as yet undefined mechanisms. Within the confines of the complex homeostatic role hormones play in lymphocyte function, the effects of the gonads on the immune response and autoimmune disease are particularly apparent. In general, females are far more susceptible to most connective tissue diseases than males. For example, the incidence of SLE in women after puberty is 9 times that in men. There is no apparent explanation for this sex difference, but experimental and clinical studies in humans and animals tend to incriminate, at least in part, female sex hormones rather than X chromosome-associated genes. Consistently, females and castrated males, both in lower animals and in humans, have higher levels of immunoglobulin and higher specific immune responses than sexually normal males, although the direct immunosuppressive effects of testosterone or immunoenhancing effects of estrogens have not been shown conclusively. Recent findings of elevated estriol levels in SLE patients with manifestations of Klinefelter's syndrome and of failure of the castrated female monozygotic twin of a lupus victim to develop the disease suggest further that chronic estrogenic stimulation may play an important role in the prevalence of SLE in females. Indeed, although the total amount of estrogens recovered from female human SLE subjects is normal, estradiol activity may be enhanced by abnormalities in female hormone metabolic patterns. Thus, Lahita and Kunkel found that women with SLE had elevations in the 16α-hydroxylated compounds of 16α-hydroxyestrone and estriol in their serum compared with normal subjects.

As in human SLE, studies by Talal and associates in the murine SLE model of (NZB × NZW)F_1 mice implicate sex hormones as accelerating factors in autoantibody levels and the overall earlier mortality rate in females than in males. Castrated (NZB × NZW)F_1 males resemble females in that they have an accelerated autoimmune disease detectable at the age of 6 months. However, testosterone or dihydrotestosterone inhibits the onset of this autoimmune disease in female mice and in castrated male (NZB × NZW)F_1 mice following subcutaneous implantation of the androgens in Silastic tubes. On the other hand, although prepubertal castration of female (NZB × NZW)F_1 mice is without effect, estrogen administration accelerates overt disease in both males and females. The mechanisms whereby sex hormones modify disease in (NZB × NZW)F_1 mice are largely unknown, but postulated explanations include effects on antigen presentation and handling by the immune system and androgen-induced enhancement of suppressor T cell activity or of tolerance inducibility.

The accelerating effects of female factors such as estrogens is by no means applicable in all human and animal autoimmune diseases. For example, the incidence of ankylosing spondylitis, possibly an autoimmune disease, is higher in males than females. More-

over, in murine models of SLE other than the NZB/W, the females are not hardest hit. For example, in the MRL/l mouse, sex hormones appear to have little effect, since the females die only slightly earlier than the males; and, contrastingly, BXSB males develop the disease much earlier (50% mortality rate at 6 months of age) than the females (50% at 18 months). In this last strain, the male sex-determined accelerated autoimmunity is Y chromosome-linked and not hormonally mediated. This conclusion is based on the following: (1) Castration of males has no effect on the course of the disease; (2) the disease is inherited in a Y-linked or holandric (father to son) fashion in F_1 crosses of BXSB males with other autoimmune strains; and (3) transfer of early, severe lupus by male BXSB bone marrow or spleen cells is independent of the lethally irradiated BXSB recipient's sex. However, recent studies with partially inbred congenic NZB/BXSB and NZW/BXSB mice have suggested that full expression of autoimmunity requires not only the Y chromosome but also one or more BXSB autosomal genes. Interestingly enough, a human counterpart of BXSB male-predominant disease was recently described by Lahita and Kunkel in familial studies of patients with SLE. They observed that full expression of the disease predominated in fathers and sons, whereas females, despite having some autoantibodies, did not develop fully expressed SLE with associated glomerulonephritis.

VIRAL FACTORS IN AUTOIMMUNITY

Viruses are frequently associated with autoimmune diseases of humans and animals. Such infectious agents may be acquired by horizontal or vertical transmission, and they may promote autoimmune reactions by many and varied mechanisms—among them, polyclonal activation of lymphocytes, release of subcellular organelles after cellular destruction, associative recognition phenomena in which insertion of viral antigens into cellular membranes may promote reactions against preexisting self components, antigenic mimicry, ectopic induction of Ia antigens via interferons, and direct infection and thus functional impairment of certain subsets of regulatory cells such as suppressor T cells.

Among the human viruses, EBV has been most prominently considered as a cause of autoimmune diseases because of its ubiquity, persistence, and ability to act on the immune system. For example, EBV acts as a polyclonal B cell activator stimulating mitoses and immunoglobulin secretion as well as promoting autoantibody production, especially rheumatoid factor. The sera of rheumatoid patients contain an antibody that recognizes unique EBV-induced antigens (RAP—rheumatoid arthritis precipitation, and RANA—rheumatoid arthritis nuclear antigen) present in extracts of an EBV-carrying B type lymphoblastoid cell line of human origin. Furthermore, T cells from these patients have less capacity to suppress EBV-induced B lymphocyte transformation than their normal counterparts. Based on both of these findings, this virus could be involved in the pathogenesis of rheumatoid arthritis. However, seroepidemiologic studies indicate that (1) as a group, subjects with rheumatoid arthritis have the same exposure to EBV as individuals without the disease; (2) antibodies to EBV-associated antigens are not a unique characteristic of such patients but can be found, albeit less often, in normal persons; (3) no evidence proves that EBV enters the joint space; (4) arthritis does not accompany certain EBV syndromes, such as infectious mononucleosis; and (5) in patients with early (under 6 weeks) rheumatoid arthritis, there is no elevation of anti-EBV antibodies, and in one patient with early rheumatoid arthritis no serologic evidence of prior EBV infection or antibody to RANA antigen was detected. These findings refute a primary role for EBV in the etiology of rheumatoid arthritis.

A variety of other viruses have been incriminated as causative agents of autoimmune diseases in humans: myxoviruses, hepatitis viruses, cytomegaloviruses, coxsackieviruses, retroviruses, and others. Most of these viruses induce autoantibodies during natural infections and also autoimmune disease-like immunopathologic characteristics such as vasculitis and glomerulonephritis— which, however, appear to be caused primarily by specific virus-viral antibody immune complexes rather than by autoantibody-antigen complexes. An oncornavirus-associated origin of human SLE, although claimed, has not been proved. Thus, although particles resembling viruses have been observed in lymphocytes and kidneys of SLE patients, it is generally agreed that these particles are artifacts or cell structures that have no relationship to viruses. Moreover, isolation of C type viruses or antigens thereof from spleens and placentas of SLE patients is disputed; C type virus antigens, in spite of claims to the contrary, are not conclusively established as components of glomerular immune complex deposits, and repeated attempts to demonstrate specific antibodies against C type viruses in sera of SLE patients have failed. Although increased titers of antibodies against certain viruses, such as measles, have been found in sera of SLE patients compared to normals, this finding has been considered nonspecific—the result of these patients' hypergammaglobulinemia. Despite these negative results, the search for a virus associated with human SLE and other autoimmune diseases continues. Thus, very recently, a small virus resembling a parvovirus was incriminated in the pathogenesis of rheumatoid arthritis. Interestingly, patients with SLE have been found to express, in serum, increased levels of a unique interferon (IFN α)—produced normally by leukocytes in response to viral and nonviral stimuli—which, unlike regular IFN α, was inactivated at pH 2. Although increased interferon levels have been observed by some investigators to be more prevalent in patients with active SLE, others have not been able to relate any individual serologic or clinical marker of

disease to the presence of interferon. Thus, the importance of this finding is unclear at this time.

For obvious reasons, investigative studies of the relationship between viruses and autoimmune disease are best conducted by using animal models that have characteristics in common with the disease in humans. Thus, a viral origin has been established in the disease of Aleutian minks involving a small DNA virus; in equine infectious anemia associated with a transmissible C type viruslike agent that has been isolated; and possibly in canine SLE comprising anti-DNA antibodies, rheumatoid factor, anti-red blood cell antibodies, hypergammaglobulinemia, LE cells, and C type virus.

Experiments have also been performed to determine the role of viruses and their antigenic components in the pathogenesis of autoimmune disease in murine SLE models. Initial reports that NZB disease could be transferred to normal mice with cell-free extracts and filtrates of NZB splenocytes were unconfirmed. Subsequent research demonstrated that NZB mice express infectious xenotropic type C RNA virus throughout life and in high titer. The virus is not found in the mouse itself but becomes apparent after cocultivation of its tissue homogenates with heterologous cells such as those from cats. The correlation between high titers of xenotropic virus production in vitro by tissue homogenates of NZB mice and autoimmunity then suggested a cause-and-effect relationship. This concept was strengthened when viral antigen-antiviral antibody immune complexes were recovered from renal lesions in NZB and (NZB × NZW)F_1 mice. The failure to transmit autoimmune disease with cell-free filtrates was explained by the fact that xenotropic C viruses cannot productively infect mouse cells, only cells of heterologous species. Subsequently, the magnified expression of xenotropic virus in NZB mice was established by Datta and Schwartz as a genetically determined trait controlled by 2 independently segregating, autosomal dominant loci (Nzv-1 and Nzv-2). This demonstration has facilitated genetic analysis of the relationship between xenotropic virus and autoimmunity. The hybrid chosen for study of this relationship was NZB/SWR, because NZB are homozygous for the dominant alleles of viral expression whereas SWR are homozygous for the recessive alleles and do not spontaneously develop autoimmune disease. Analysis of the F_1, F_2, and back-cross progeny of NZB × SWR mice demonstrated the following: (1) Some progeny whose tissue homogenates express titers of xenotropic virus as high as those of the NZB parent fail to develop signs of autoimmunity; (2) virus-negative offspring from these crosses still develop autoantibodies; (3) the phenotypic expression of virus does not correlate positively with the incidence of glomerular lesions; and (4) levels of the viral antigen gp70 do not correlate with the development of nephritis in these crosses. More recently, no correlation between autoimmunity and xenotropic or ecotropic C type virus levels has been observed by Datta and associates in recombinant lines of NZB and C58 mice.

Gp70, the major glycoprotein component of the envelope of type C RNA viruses, is found in tissues and sera of virtually all mice. Structural studies of serum gp70 indicate that it is the same in all strains and resembles the gp70 of the NZB xenotropic virus. Its presence is independent of the expression of complete retrovirus particles, and it appears to be produced primarily in the liver. Gp70 has also been implicated in the pathogenesis of lupus nephritis in spontaneous murine models, because of the high concentrations of gp70 in the sera of these mice and because gp70 is deposited in diseased glomeruli along with specific antibody, complement, and nuclear antigens and antibodies. However, similarly high levels of identical gp70 have been detected in several immunologically normal strains of mice, indicating that gp70 per se is not the factor that determines disease expression. In contrast to normal strains of mice, only SLE mice produced antibodies against xenotropic gp70 and had gp70-anti-gp70 complexes in their sera. The gp70 that participates in the formation of immune complexes was no different antigenically or structurally by tryptic peptide map analysis from the free xenotropic gp70 found at varying levels in sera of all murine strains. Thus, the high serum levels of xenotropic gp70 apparently do not in themselves cause murine nephritis; rather, the unique ability of autoimmune mice to respond to this autoantigen is the critical factor. To summarize: These studies indicate that although xenotropic virus and viral antigens may participate secondarily in the formation of immunopathologic lesions, they are not a primary cause.

Chronic viral infections may have a secondary role in autoimmune diseases if their superimposition on an autoimmune genetic background accelerates autoimmunity. For example, lymphocytic choriomeningitis virus, polyoma virus, and retrovirus infections have all been observed to induce or elevate antinuclear antibodies and SLE-like disease in mice. Although these viruses probably act in part by causing antivirus antibody and immune complexes to form, their stimulation of antinuclear and other autoantibody formation must be considered as potential means of enhancing SLE. Neonatal lymphocytic choriomeningitis virus infection changes the 50% mortality rate point caused by SLE-like disease from 16 months to less than 5 months in the NZB female, from 18 to 9 months in the BXSB female, and from 17 to 12 months in the MRL/Mp-+/+ mice. In contrast, normal C3H and SWR mice infected neonatally with lymphocytic choriomeningitis virus and examined from birth to 2 years of age do not develop the fatal SLE-like disease.

In general, viruses—via their polyclonal B cell-activating potential, their cytolytic capacity, their possible tropisms for certain subpopulations of lymphoid cells, and their possible capacity to associate with and convert autoantigens to foreign antigens—may induce aberrant responses and autoagression. However, as in the case of the 2 other immunostimulators (LPS, lpr gene) discussed in other sections of this chapter, an ap-

propriate predisposed genetic background is required for expression of immunopathologic manifestations.

CONCLUSIONS

The study of autoimmunity brings together a fascinating diversity of fields, including immunology, pathology, endocrinology, virology, genetics, and molecular biology. This chapter describes some of the intellectual terrain that has been explored in the search of what we know at present about autoimmune disorders and the reasons for their occurrence.

The role of self recognition in the immune system has been discussed in light of recent experimental data. These findings indicate that under certain conditions, recognition of self-determinants is not totally forbidden or harmful. Thus, before T cells can differentiate to become effector cells, they must recognize both foreign antigen and self-MHC determinants. Furthermore, homeostasis of immunity and control of immune responses appear to involve a complex web that interconnects all lymphocytes and their antigen receptors via self V-anti-self V domain determinants, the so-called idiotype-anti-idiotype network. Since autorecognition is apparently a normal event in a functioning immune system, the proposal that autoimmune diseases may result in part from an imbalance or aberration of complementary idiotypic-anti-idiotypic responses is reasonable.

Despite the presence of these functionally important normal forms of autorecognition, the fact remains that an individual generally does not respond to a detectable degree against most of its own constituents. The process of inducing tolerance to self, as suggested by experimental studies of tolerance induction to foreign antigens, could be attributed to numerous mechanisms of which the most acceptable are clonal silencing and engagement of suppressor T cells, the latter mechanism presumably acting by the idiotype-anti-idiotype circuit. It is generally agreed that immature immunocytes are much more susceptible to tolerance induction than mature cells. Since the turnover of B cells is very rapid, one can logically conclude that the process of inducing tolerance to self is a continuous event that occurs repeatedly throughout the life of an individual whenever primitive cells with a genetic commitment to self-reactivity emerge from the hematopoietic organs, where they have the opportunity to meet self antigens in situ. Foreign antigens do not usually induce tolerance, because they pass through a succession of lymph nodes where they have optimal opportunity to meet tolerance-resistant mature immunocytes.

What causes failure of the phenotypically apparent self-tolerance mechanism is unknown. However, genetic factors combined with a variety of primary or secondary immunologic abnormalities—as well as hormonal abnormalities and infectious agents such as viruses—may promote the development of autoimmune diseases. The abnormal genes responsible for expression of autoimmune syndromes have not yet been identified, but studies in animal models of SLE have shown that many independently segregating genes could be responsible for the formation of the various autoantibodies. However, a close correlation among various autoimmune traits shown to have association with survival (ANA, anti-DNA, anti-gp70, high IgG levels) suggests either genetic linkage or a pleotropic gene action. Despite the observed (minimal) association between most autoimmune diseases of humans and certain MHC and immunoglobulin genes, the genes controlling expression of autoantibodies in murine lupus have not been closely linked to any particular H-2 type or allotypic marker. Further studies aiming at the precise chromosomal assignment and location of autoimmunity-promoting genes will be of extreme importance in our attempts to understand the pathogenesis of these disorders and possibly to interfere with their progress through genetic engineering techniques of the future.

The etiologic events as well as the actual immunologic abnormalities leading to autoimmunity remain ill-defined—especially those of organ-specific autoimmune diseases, for which few animal models are available. Studies in humans and mice with SLE, perhaps the most extensively studied autoimmune disease, have produced a long list of abnormalities at both the B and T cell levels. Originally, the prevailing view was that the characteristic B cell hyperactivity of this disease was secondary to suppressor T cell abnormalities. However, this explanation now seems doubtful. Transplantation experiments between NZ or BXSB SLE murine strains and their normal congenic or histocompatible counterparts indicate that hematopoietic stem cells and precursors of B cells can transfer the disease. This indicates that a primary lymphoid cell defect is the responsible agent for this disease and effectively rules out abnormal autoantigens or other nonlymphoid environmental factors as primary participants. The B cell abnormality is not expressed autonomously but requires helper T cell-derived accessory signals that play a role in B cell proliferation and differentiation.

Whatever the basic genetic and molecular immunologic defects, such defects may not become overt until late in life unless accelerating factors are superimposed. These accelerating factors may be endogenous and genetic in nature. One example is the Y chromosome-linked accelerating genes of BXSB mice that predispose to a much earlier development of SLE in male than in female mice; another such example is the lpr gene of MRL mice that predisposes, by inducing proliferation of helper T cells, to the earlier expression of disease in MRL/Mp-lpr/lpr mice compared to late-disease-developing congenic MRL/Mp-+/+ mice that lack the lpr gene. Accelerating factors of autoimmunity may also be female hormones and some exogenous factors such as viruses and bacteria that may activate polyclonally self-reactive B cells. However, for these immunostimulators or accelerators to induce immunopathologic manifestations, an appropriate predisposed genetic background is required.

In this very complex subject of autoimmunity, all aspects of modern immunology are involved. By using the framework outlined above, additional studies, especially of related genetic and molecular abnormalities, should provide a quite complete description of these diseases in the not too distant future.

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Immunity in Mucosal Tissues

12

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The epithelial surfaces of the body play a key role in the interaction between the external and internal environments and in this way extensively influence the immune system. While the epithelium serves as a barrier to antigenic material, it is by no means impenetrable, and the underlying tissues are constantly bombarded with numerous challenges such as microbial and food antigens in the lumen of the intestine. Immune responses must be effective and appropriate: They must limit infections but must also be closely regulated so as not to compromise the integrity and function of the fragile mucosal tissues they protect.

It has long been known that the presence of antibody in local secretions correlates better with protection against pathogenic organisms than serum antibody. With the discovery by Tomasi and Hanson that the major antibody in external secretions was IgA in a unique secretory configuration (**secretory IgA; sIgA**) came the belief that sIgA was completely responsible for immune protection of mucosal surfaces. While it is still true that IgA remains the best marker of local mucosal responses, other humoral and cellular mechanisms must also be considered in assessment of mucosal resistance.

Thus, in addition to their environment and the predominance of IgA, mucosal immune responses have been shown to consist of other unique properties, including their sites of antigen presentation, regulatory cells, and mast cells. Despite some of the differences between systemic and mucosal immune mechanisms, it is clear that local immune mechanisms interact with responses which occur at other mucosal and systemic sites. These interactions may be mediated by cells, lymphokines, or possibly neuroendocrine signals triggered by exposure to antigen. Thus, mucosal antibody and cell-mediated immunity may provide local resistance to bacteria, viruses, parasites, toxins, and allergens. However, it is possible to prime or even to suppress a systemic response by sensitization of mucosal tissues such as the gut.

This chapter will discuss a variety of concepts regarding mucosal immunity, with emphasis on the intestine. The distribution of various **mucosa-associated lymphoid tissues (MALT)** will be described and the processes of antigen uptake, immune regulation, and humoral and cellular immunity reviewed. We will also attempt to illustrate how mucosal immune reactions are integrated with the rest of the immunologic apparatus and how the responses may be involved in the prevention or pathogenesis of diseases of mucosal surfaces.

DISTRIBUTION OF MALT

Throughout the intestine, the **gut-associated lymphoid tissue (GALT)** consists of 2 types of lymphoid aggregates. The first is referred to as Peyer's patches, composed of multiple lymphoid aggregates; the second consists of isolated lymphoid follicles—often referred to as solitary lymphoid nodules—which are found predominantly in the colon. Peyer's patches develop in utero and increase in size and number with exposure to antigen. They have a defined microstructure, including a central B cell-dependent follicle and T cell-dependent regions adjacent to the follicle (Fig 12–1). The lymphocytes in Peyer's patches are quite heterogeneous, as they include B cells expressing IgM, IgG, IgA, and IgE plus various regulatory and cytotoxic T cells. Peyer's patches also contain specialized macrophages believed to act as antigen-presenting cells. The patches themselves are covered by a specialized lympho-epithelium (follicle-associated epithelium) consisting of so-called M cells. M cells selectively pinocytize and phagocytize particles and transfer this material to the follicle. M cells originate in the crypts and migrate to the dome of the aggregate, where they are transformed into Ia (class II MHC) antigen-bearing cells. Similar epithelium is found in the chicken bursa of Fabricius, an important site for generation of B cells in chickens. There are no afferent lymphatics to Peyer's patches. However, efferent lymphatics lead to mesenteric lymph nodes and the thoracic duct.

Lymphoid cells are also seen diffusely scattered throughout the lamina propria and between the absorptive epithelial cells (intraepithelial compartment). The size of these compartments and the dense concentration of cells within them make the lamina propria and epithelium a major source of lymphoid cells in the intestine. Whereas the healthy lamina propria contains many different cells such as T and B lymphocytes, macrophages, polymorphonuclear cells, and mast cells, the distribution can be markedly altered in inflammatory states. In the human small intestinal lamina propria, an average of 85% of B cells contain IgA, 5% IgG, and 10% IgM. Here the majority of the

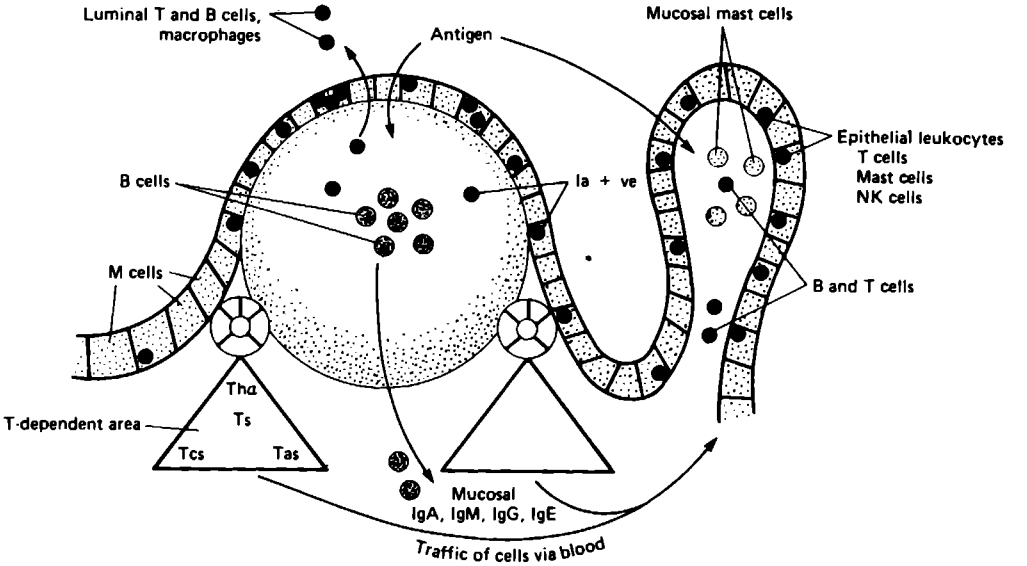

Figure 12–1. The distribution of cells within GALT and their interaction with antigen. Luminal antigens may enter Peyer's patches via M cells or may penetrate the absorptive enterocyte layer. Subsequently, Ia$^+$ antigen-presenting cells (macrophages, possibly enterocytes or B cells) stimulate local B and T cell responses, depending on the equilibrium among helper T cells (Th), suppressor T cells (Ts), and contrasuppressor (Tcs) or antisuppressor (Tas) cells. Stimulated B and T cells leave Peyer's patches and selectively localize elsewhere in the intestine, and to a lesser degree other MALT. B and T cells are found in lamina propria with other leukocytes, whereas no B cells are found within the heterogeneous intraepithelial leukocyte compartment. (Reproduced, with permission, from Ernst PB et al: Oral immunization and tolerance. In: *Immunology of the Gastrointestinal Tract and Liver*. Jones R et al (editors). Raven Press. [In press.])

T cells have the phenotype associated with the helper cell subset.

The intraepithelial compartment consists almost exclusively of mononuclear cells; it lacks macrophages but contains mast cells and their precursors, which become more numerous in many diseases in the intestine. Of the lymphocytes in the epithelium, only T cells are seen. Most of these intraepithelial leukocytes express at least one antigen associated with cytotoxic/suppressor T cells. Paradoxically, most of these do not express other pan-T cell markers, and they contain intracytoplasmic granules resembling those seen in large granulated lymphocytes. While some of the granulated intraepithelial leukocytes possess natural killer (NK) activity, the majority do not, and their function is not known.

Lymphocytes and macrophages are also found in small numbers in the lumen of the intestine. Increased numbers of luminal lymphocytes are characteristic of viral diarrheas, whereas polymorphs predominate in bacterial diseases.

Bronchus-associated lymphoid tissue consists of lymphoid aggregates strikingly similar in morphology to those seen in the intestine. They possess a specialized follicle-associated epithelium that contains M cells with the same propensity for acting as selective sites of soluble and particulate antigen sampling. Although less clearly defined, they appear to have discrete T and B cell-dependent areas and, like the gut, have no afferent lymphatics but possess efferents that drain to local lymph nodes.

Other mucosal tissues in the body include the middle ear, parts of the urogenital tract, the mammary gland, the conjunctiva, and the salivary glands. Few comparisons have been made between these tissues and the lymphoid tissues described in the gut or lung. While the presence of sIgA characterizes the secretions that bathe these tissues, complete analysis of their lymphoid tissues and components in the MALT has not been made. Lymphoid follicles have been described in some of these tissues as, and are said by Ham to be characteristic of, "wet epithelia." All of these tissues have a similar direct exposure to the external environment. More research is required to define the sites of antigen uptake (if any) and to determine whether or not the epithelium in this site is specialized. From this point of view, it is interesting that under the influence of lymphokines, intestinal—and, to an unknown extent, other epithelia—can express Ia antigens and so may acquire antigen-presenting capability.

ANTIGEN UPTAKE & STIMULATION OF MALT

In 1927, Besredka reported on the successful application of his experimental work in laboratory animals

to the oral immunization of thousands of humans against dysentery and typhoid fever. Despite this early success and some more recent ones such as poliomyelitis and typhoid fever, we are still somewhat limited in our ability to manipulate mucosal immune responses to restrict the impact of mucosal infections in human and veterinary medicine.

The epithelium overlying mucosal tissues does not act as a complete mechanical barrier to macromolecules or particulate antigens. The sites of penetration include the villous extrusion zones for epithelial cells, through or between epithelial cells or across the specialized lympho-epithelium such as the M cells. Thus, the potential for interaction between antigen and the lymphoid cells spread throughout the mucosa is immense. Since the specialized epithelium is so efficient at selective sampling of antigen, this would focus the stimulation of the immune system to sites such as MALT or its draining lymph nodes. This restriction of antigen uptake in health may help regulate local immune responses by limiting antigen absorption and selecting for the appropriate regulatory mechanisms that exist in Peyer's patches. In the course of pathologic processes involving an epithelial surface, the permeability may be altered. This could also lead to an augmented local responsiveness to this increase in antigen dose.

Various factors may influence antigen uptake and reactivity, and no uniform principles applicable to all species have yet emerged. In several species but not humans, adrenal corticosteroids are known to effect "closure" of the intestinal epithelium, which for a variable time after birth selectively or nonselectively transports luminal macromolecules. In addition, testosterone appears to affect the uptake of antigen in chicken bursa.

Relative digestibility of antigen in passage through the stomach and intestine is also important. The chemical nature of the antigen is crucial, since oral cholera toxin is such a good immunogen and can even enhance the reactivity to other antigens conjugated to it and therefore shows promise as an adjuvant. Vitamin A and bile salts have been shown to be effective local adjuvants, presumably through their ability to influence membrane activity. Polyornithine and polylysine as well as detergents also apparently act as adjuvants. Other influences such as the flora also affect immune reactivity. Thus, streptomycin and lysozyme affect responsiveness perhaps by altering flora. The study of these and other local adjuvants and immune modulators is receiving considerable attention because of its practical importance.

The initial priming of mucosal immune responses usually occurs either in MALT or draining lymph nodes. MALT serve to amplify memory responses through their antigen-focusing activity. However, parenteral immunization in an individual rarely produces mucosal immunity unless the antigen reaches the mucosa (ie, live virus vaccines) or unless prior exposure via the mucosal route has occurred. Dendritic macrophages may migrate from MALT to lymph nodes, but it is not known if these cells act strictly locally or elsewhere in the body as a result of a selective migratory pattern. This is a distinct possibility, since veiled cells of intestinal origin with accessory cell function have been identified in thoracic duct lymph after surgical removal of the mesenteric lymph nodes.

Peyer's patches contain effector T and B cells plus a wide array of regulatory T cells, including helper cells, IgA-specific regulatory cells, and contrasuppressor or antisuppressor cells involved in elaborate regulatory circuits or cascades. Following the stimulation of T or B cells, activated lymphoblasts are believed to exit from these sites via the efferent lymphatics to regional draining lymph nodes. These cells then enter the thoracic duct lymph and are subsequently distributed throughout the body via the blood. Mucosa-derived lymphoblasts selectively migrate to or accumulate in the organ tissue from which they were derived. However, they also possess a relative selectivity for eventual localization in other mucosal sites. Thus, cells stimulated in MALT may then return to seed the rest of the intestine as well as other mucosal sites where they may perform their role in resistance. This means that there is an exchange of information mediated by the migration of antigen-specific effector lymphocytes from one site (ie, Peyer's patches in the gut) to other sites in the gut and other tissues such as the lung, breast, or reproductive tract. This may help explain the observation that immunization of one mucosal surface often leads to the production of antibodies with the same specificity on other surfaces and supports the argument for the presence of a common mucosal immune system. This theory suggests that cells primed in a mucosal site would seed other mucosal tissues, where, if they meet the same antigen, they would proliferate and amplify a broad base of mucosal protection. It also suggests that the best means of producing widespread mucosal immunity would be to immunize orally, because of the amount of intestinal lymphoid tissue.

In addition, several factors have been postulated to contribute to the accumulation of mucosal lymphocytes at specialized sites. There may be molecules on lymphocytes complementary to others on vascular endothelium that attract these cells to particular tissues. In addition, IgA may allow specific binding to structures in the mucosal tissues after exit from the vascular compartment. Alternatively, the process may be more random and depend in large measure upon the blood supply to a particular organ. In the breast and female genital tract, sex hormones clearly influence cellular localization. Protein malnutrition and vitamin A deficiency also depress this localization through unknown effects. The amounts of antigen and mitogens such as lipopolysaccharides at these sites may selectively stimulate mucosal populations to expand and thus amplify any effect of selective migration or trapping.

MUCOSAL ANTIBODY RESPONSES

The predominance of IgA in the external secretions is explained in part by the selective concentration of IgA-producing immunocytes at the external mucosae. The mechanism responsible for the selective concentration of IgA-producing immunocytes is not fully understood but may involve specific recognition mechanisms operating at the vascular endothelium supplying blood to mucosal tissue. As described previously, IgA-committed cells form part of a recirculating pool that may be stimulated locally to produce antibody or may have emigrated from a distant mucosal site where they were previously primed by antigen.

Once IgA is secreted by mucosal immunocytes in the lamina propia, it is selectively transported across the mucosal epithelium to function in the external secretions. The selective secretion of IgA is promoted by certain structural features of this immunoglobulin that allow it to bind to a specific transport receptor termed **secretory component (SC).** IgA resembles other classes of immunoglobulins being made up of 4-chain monomer subunits each with 2 antigen-binding sites (Fig 12–2). The α heavy chain has an extension at its C-terminus that contains a cysteine residue, allowing it to form disulfide-linked polymers of the 4-chain monomer subunit. J chain, a 15,000-MW polypeptide also synthesized by plasma cells, promotes the formation of **polymers of IgA (pIgA).** Formation of polymers appears critical to allow IgA to bind to the mucosal transport receptor (SC).

The mechanism of transport of pIgA has been well studied and is one of the best examples of the nondegradative transcellular transport of proteins through cells. Secretory component, which mediates the transport of pIgA, is a 90,000-MW protein expressed as an integral membrane protein on the basolateral membrane of mucosal epithelial cells. This receptor is apparently continuously endocytized and transported across the epithelial cell and secreted at the apical membrane into the mucosal secretions as a 70,000-MW soluble fragment. The transport of pIgA occurs following its synthesis and secretion by immunocytes in the lamina propia. Polymeric IgA binds with high affinity to SC on the epithelial cell and is transported to the mucosal secretion bound to its receptor in the form of a complex (SC-pIgA; Fig 12–2). Mucosal secretions therefore contain a mixture of secretory IgA and free SC, except in patients with immunoglobulin deficiency where only free SC is found. IgM can also be transported by this process, and in individuals deficient in IgA, a compensatory increase in secretory IgM containing SC often is found.

This transport process is unique in that it promotes the uptake and transport of a large protein without entry into the lysosomal degradation pathway. In addition, the SC must be continually resynthesized, since, unlike many transport receptors, it is not recycled. In some species (eg, rat and rabbit), SC is also expressed in large quantity on hepatic parenchymal cells, and this allows recovery of pIgA from blood for redistribution to bile and finally to intestinal secretion. This latter process does not occur to a significant extent in humans. However, selective secretion of pIgA may occur to a small extent via the biliary epithelium.

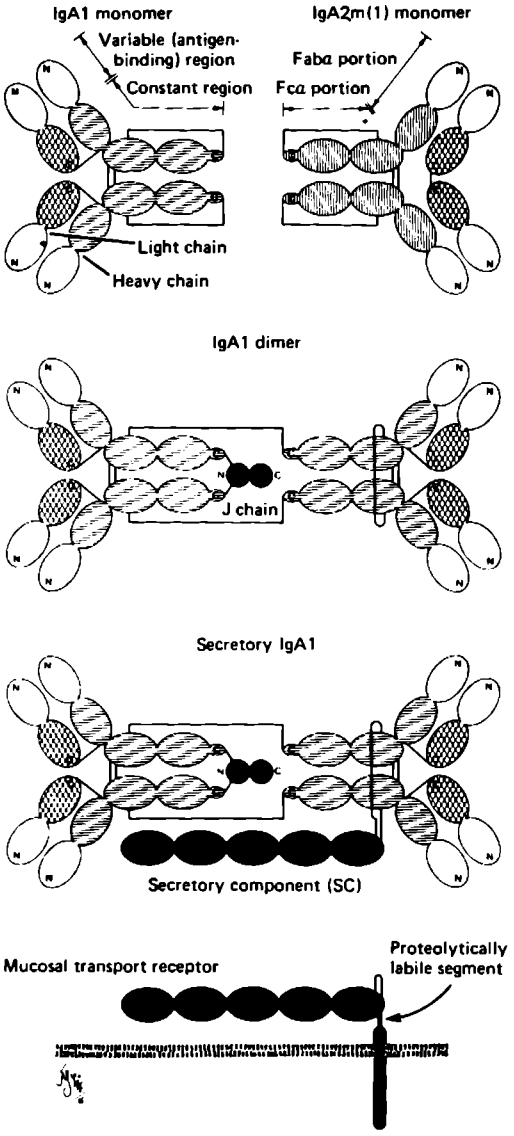

Figure 12–2. Structural properties of IgA. IgA monomer has 2 subclasses (IgA1 and IgA2) that can form dimers via J chain. Following the binding of pIgA to the SC on epithelial cells, sIgA is transported to the luminal membrane, where SC is cleaved and sIgA released into the secretion. (Reproduced, with permission, from Underdown BJ, Schiff JM: Immunoglobulin A: Strategic defense initiative at the mucosal surface. *Annu Rev Immunol* 1986;4:389.)

This mechanism of pIgA transport is operative on the epithelial surfaces of many mucosal tissues that can clear pIgA from serum produced at other mucosal sites. Moreover, pIgA bound to antigen may also be

transported across epithelia, a circumstance that would limit the exposure of a host to excessive amounts of antigen which may have leaked across the epithelium. Such IgA-antigen complexes may also circulate to other mucosal surfaces, where, in association with their clearance, they may help stimulate local immune responses.

Secretory IgA appears to have unique properties that may explain its predominance at the mucosae (Table 12–1). Both the polymeric nature and the addition of SC confer on secretory IgA considerable resistance to proteolysis. IgA is a poor activator of the classic pathway of complement. Indeed, some investigators have reported that sIgA antibodies can competitively inhibit the complement-activating activity of IgG antibody. It has been hypothesized that IgA may lack complement-activating activity in order to attenuate the inflammatory potential of IgG antibodies at the sensitive epithelial surface. IgA, on the other hand, has been reported to activate the alternative pathway of complement, and this may provide some protective function as well as promote inflammation to some extent. Receptors for IgA have been described on inflammatory cells that may promote the destruction of cellular pathogens (eg, bacteria, parasites) by antibody-dependent cell-mediated cytotoxicity.

IgA antibodies may exert their microbial function primarily by binding to antigenic epitopes on microorganisms. This binding restricts their mobility or prevents their binding to mucosal epithelium. The functional requirement for IgA is clearly not absolute, since many IgA-deficient individuals are apparently healthy. However, the fact that IgA-deficient individuals have an increased prevalence of mucosal infections, atopy, autoimmune diseases, and absorption of food antigens clearly indicates that IgA plays a role in the multicomponent immune system.

Little is known about the function of IgA in blood. In humans, circulating IgA is 10% polymeric and 90% monomeric, with significant amounts of both types apparently being produced in the bone marrow. The role serum IgA plays in immune protection is uncertain, but it may be important in antigen clearance and immune regulation. IgA has been implicated in several pathologic conditions such as dermatitis herpetiformis, IgA nephropathy, anaphylactoid purpura, and bullous pemphigoid. (See Chapter 29.)

IgA exists in 2 subclasses—IgA1 and IgA2—and the IgA2 subclass exists in 2 allotypic forms: A2m(1) and A2m(2). The IgA2 subclass, which is lacking a proteolytic-sensitive site in the α-chain hinge region, is enriched in the external secretions, and this may represent an evolutionary adaptation to counter a series of proteolytic enzymes synthesized by mucosal bacteria that specifically cleave IgA1 antibodies. There are few data on significant differences in transport or function between the IgA subclasses. In addition to deletion in the α-chain hinge region, IgA2m(2) molecules have their light chains disulfide-bonded to each other rather than to the α chains, but no function has been attributed to this difference.

Table 12–1. Functions of IgA.

Resists proteolysis
Blocks–
Uptake of antigen
Bacterial or viral attachment
Complement-dependent lysis
Toxin activity
Allergic reactions
Limits inflammation–
Complement fixation minimal by classic pathway
May act by alternative pathway
Limits penetration of antigen
Helps clear absorbed antigen
Blocks bacteriolysis
Binds to regulatory T cells, which may help control level of IgA
Binds to different leukocytes and may be involved in antibody-dependent cell-mediated cytotoxicity

IgM & IgG

Although it is usual to emphasize IgA in discussions of mucosal immunoglobulins, it is not appropriate to ignore the other classes of antibody. Cells committed to other classes of antibody are found in bronchus-associated lymphoid tissue and other MALT. In fact, the secretions in the peripheral airways of the respiratory system contain greater IgG:IgA ratios than observed in the nose or upper respiratory tract. In addition, oral immunization is often associated with increased production of serum IgG.

The mesenteric lymph nodes have been shown to be an enriched source of intestinal IgG and IgM precursors, and these cells in the mesenteric lymph nodes show the same selective localization to the intestine as observed for IgA-producing cells. The role of these classes of antibody in the protection of mucosal surfaces is incompletely understood. They are not transported nearly as efficiently as IgA into mucosal secretions, and they are not as resistant to proteolysis, thus making them less significant in the lumen.

IgE

IgE—the major reaginic antibody—is present in the secretions of several mucosal tissues such as the nose, eyes, bronchi, and gut, with only small quantities in milk. This antibody class is synthesized by cells found in MALT. The extent to which the regulatory mechanisms described for IgE synthesis apply to mucosal IgE production is not known. Under certain conditions, oral immunization has been shown to suppress IgE production through T suppressor cell mechanisms. The migration pattern of cells producing IgE has yet to be completely defined. It is interesting that cells in MALT at birth or in the germ-free state express considerable surface IgE and that many such cells also express IgA. This indicates a role for our environment and flora in the down-regulation of IgE expression. It is likely that an understanding of these phenomena will enhance our ability to control allergic reactions to the myriad of antigens to which we are exposed via inhalation, ingestion, or contact.

MUCOSAL CELLULAR IMMUNE MECHANISMS

The mucosal cells contain a full complement of leukocytes whose distribution was discussed earlier. Within the intestine, they are well compartmentalized, with each compartment being morphologically distinct. Furthermore, the different compartments contain varying proportions of the different cell lineages, which reflects specialized function for each region. For example, the lamina propria contains T and B cells, while the adjacent epithelium lacks B cells. Clearly, the contribution of the 2 compartments to local resistance differs significantly based on the density of the different effector cells.

Cytotoxic T Cells

Following oral or intraperitoneal immunization, cytotoxic T cells can be detected in Peyer's patches, lamina propria, and epithelium. Such cells are capable of recognition of host cells with altered or nonself surface antigens and are believed to be important in resistance to tumors and viruses. It is not clear where the precursors of these cells lie waiting for the signal from the antigen. Some may be present in Peyer's patches and contact luminal antigen, while others may be diffusely spread throughout the tissue waiting to recognize local signals such as virus infection or the expression of tumor-specific antigens. Recent data suggest that precursors are indeed present in the epithelium and probably the lamina propria. The frequency of these precursors may not be as great as in the spleen, but it is high enough to lead to a significant response. This suggests that antigen presentation and stimulation of the cytotoxic T cells may occur in sites other than Peyer's patches.

NK Cells

The epithelial compartment contains a large population of granulated mononuclear cells. Similar cells have been described in the uterus, respiratory tract, and gallbladder. Their granulated cytoplasm is similar to the large granulated lymphocytes that mediate NK activity. Unprimed intraepithelial leukocytes have been shown to recognize and kill tumor targets, cells infected with enteric viruses and autologous epithelial cells. It is clear, however, that the majority of granulated intraepithelial leukocytes do not possess this activity. Preparations of cells from the intestinal lamina propria, lung, liver, and uterine tissue have also been shown to be capable of mediating some NK activity against classic NK targets. It seems that the regulation of the mucosal NK cells differs from what has been described for systemic cytotoxic cell populations.

Interestingly, most of the granulated intraepithelial leukocytes do not mediate NK activity. There is very little understanding of the function or lineage of these unusual cells. The situation is confounded further by the novel array of surface antigens expressed on some of these cells in the epithelium of mice, rats, and humans. In these instances, some of the granulated cells express the surface antigen normally associated with cytotoxic suppressor T cells (Lyt 2 in mice, OX8 in rats, and CD8 in humans), but they are peculiar since they mostly lack such pan-T cell markers as Thy-1 in mice, W3/13 in rats, or Leu-1 in humans.

The granulated cells in the uterus have been implicated as suppressor cells that may be partially responsible for the tolerance granted fetal tissues by maternal immune responses. Whether the granulated epithelial leukocytes have a similar function in limiting immune responsiveness to the luminal antigens encountered in other tissues is an interesting possibility. No such activity has been ascribed to these cells in the gut.

Intraepithelial leukocytes may also be involved in antibody-dependent cell-mediated cytotoxicity with IgA and other classes of antibody. Some intraepithelial leukocytes—or possibly cells from lamina propria—may also modulate the expression of Ia antigens by intestinal epithelial cells.

Immunization & Immune Regulation

The mucosal surfaces have been shown to contain regulatory T cells both similar to and different from those seen in spleen or peripheral blood. Gut-associated lymphoid tissue (GALT) contains T cells that regulate the production of IgA by at least 2 major pathways. Some T cells may be involved in regulating the molecular events that occur as B cells differentiate from IgM to IgA production. In this model, specific immunoglobulin gene rearrangement from IgM to IgA is induced via a soluble factor. In the second model of IgA regulation, IgA-producing cells are selectively stimulated to proliferate as a result of T cell signals. Thus, both mechanisms can select for the stimulation of IgA production. These IgA-specific helper/switch cells are found in greatest number in GALT relative to systemic lymphoid tissues.

Oral immunization has also been associated with the stimulation of helper cells that facilitate the production of other classes of immunoglobulin, including IgM or IgG. In addition to the presence of helper cells in GALT, suppressor T cells have been described. Following oral immunization, suppressor cells specific for IgG and IgM but not IgA have been shown to migrate from Peyer's patches to mesenteric lymph nodes and enter the systemic compartments where they can inhibit systemic immune responses to parenteral immunization. This phenomenon is referred to as **oral tolerance** and is an excellent example of how mucosal and systemic immune mechanisms interact. The mirror image of oral tolerance has also been described, whereby parenteral immunization has been shown to inhibit proliferation of antigen-specific antibody-producing cells in the intestine. This has dramatic implications for the design of immunization protocols, since attempts to induce mucosal immunity with parenteral inoculations may be thwarted by these regulatory pathways.

Immunity in the respiratory tract, mammary gland, or parts of the urogenital tract can also be induced following the oral administration of antigen. This may be

a result of the circulation of antigen, antibody, or immune cells. This mechanism is effective at dissemination of immunity and has been shown to play a critical role in protection of the neonate by the mother's colostrum or milk. Oral vaccination of the mother has been successfully used in veterinary medicine to protect neonates from enteric infections as a result of antibodies in the milk. There is also evidence that immunity on mucosal surfaces can be induced by direct topical stimulation. Systemic routes of immunization are often ineffective at stimulation of a protective response on these surfaces, but they may help to prime these sites or amplify local responses to mucosal antigen.

Other regulatory cells also exist in the MALT and include the contrasuppressor and antisuppressor pathways. To date, it is not clear if these are overlapping or independent regulatory networks, but they both have been identified in GALT. These models of immune regulation comprise an interactive series of T cells and provide another explanation for the control of some immunologic reactions observed in mucosal tissues, including the selection for IgA. IgA-specific suppressor cells that increase in number as IgA levels increase have been described in the spleen and may help limit the production of this class of antibody following stimulation at mucosal surfaces.

OTHER CELLS

Mast Cells

Mast cells in the intestinal lamina propria of rats possess histochemical properties different from peritoneal or connective tissue mast cells, and this had caused them to be described as "atypical." This is another example of how mucosal cells differ from their systemic counterparts. The distinctions between different mast cell populations can be based on histochemical properties, density of Fc receptors for IgE, responses to different antiallergic compounds, or chemistry, including content of enzymes, proteoglycans, and mediators such as histamine, prostaglandins, and leukotrienes.

Hyperplasia of mucosal-type mast cells in the intestine has been shown to be T cell-dependent and due to lymphokines such as interleukin-3. For some time it was not clear whether such hyperplasia as occurs in nematode infections and inflammatory bowel disease was due to the differentiation of local precursors or to their influx from the bone marrow via the blood. Recently, it has been shown in mice that the lamina propria and epithelium contain 10 times as many mast cell precursors as the bone marrow and 100–1000 times as many as the spleen, peripheral lymph nodes, or blood. This high mast cell precursor frequency suggests that a great deal of the hyperplasia observed during inflammatory processes is due to the differentiation of local precursors.

Mast cells have been shown to have a number of effects (mostly harmful) on the integrity and function of mucosal tissues. They contain an array of potent mediators which, when released, can cause damage to the epithelium and alterations in permeability and electrolyte transport. They can also cause contraction of smooth muscle in the respiratory, genitourinary, and gastrointestinal tracts. To date, the evidence regarding their protective function has been less convincing, but they may be involved together with IgE in the rejection of parasites.

Macrophages

As described previously, the antigen-presenting cells in mucosal tissues have been only superficially characterized, although macrophages from the lung and gut have been isolated and described. Macrophages derived from mucosal tissues are present in the draining lymph and clearly reach other sites. Whether they display the selectivity to migrate to other mucosal tissues or systemic sites and present antigen in those sites remains a possibility to consider.

Goblet & Epithelial Cells

For many years, goblet cells have been recognized as mucus-secreting cells that play a role in resistance to infection. These cells can undergo a hyperplasia that appears to be dependent on T cells in some cases. Goblet cells secrete mucus under the influence of the nervous system but also as a result of immune complex formation and mast cell degranulation. The nervous system may also influence mast cell degranulation and thereby affect goblet cell secretion and its role in protection against parasites and other organisms. The expression of glycoproteins on epithelial cells has also been shown to be influenced during an immune response, and this could affect their ability to function or exclude macromolecules.

INFLUENCES ON MUCOSAL IMMUNE REACTIVITY

The aim of understanding mucosal immunity is to achieve the successful manipulation of immune responses that may take part in the prevention or pathogenesis of mucosal disease. Much of the discussion in this chapter has emphasized the unique properties of mucosal immune responses, and some of these must be considered if one wishes to enhance immunity in these sites (Table 12–2). Clearly, the route of antigen administration can have profound effects on intestinal immunity, as can the chemical nature of the antigen and adjuvants, the dose, the immune status of the host, and the frequency and timing of immunization.

Mucosal immune activity is greatly affected by external influences such as diet, microbial flora, and hormones. Many immune reactions are impaired when an individual is affected by malnutrition, and those in mucosal tissues are no different. For example, deficiency of vitamin A or protein/calorie malnutrition decreases the efficiency with which lymphocytes localize in mucosal tissues. Different microbial organ-

Table 12–2. Influences on mucosal immunity.

Antigen
Route of administration
Dose, frequency
Adjuvants
Cellular tropism and attachment
Structure
Host
Immune status
Age
Nutritional status
Permeability of epithelium
Genetic background
Concurrent disease or immunologic stimulation
Neuroendocrine system
Flora
Diet
Drugs

isms can also directly affect mucosal immune regulation. Lipopolysaccharide is a potent B cell mitogen and has a number of direct effects on the helper and suppressor cell activities in the gut.

The immune system can influence physiologic processes such as epithelial cell function and goblet cell hyperplasia, and this is reciprocated by influences from neuroendocrine tissues on lymphoid cells. Various neuropeptides have been shown to affect mast cell degranulation, traffic of mucosal cells, and B and T cell responses. Thus, the mucosal immune system not only interacts with the rest of the body through lymphoid cells but may also exchange information using the far-reaching neuroendocrine pathways.

To a greater or lesser extent, mucosal and systemic immune responses are dramatically affected by the external environment. Furthermore, the maintenance of the physiologic integrity of the mucosa is complex but depends in large measure on local immune responses. An understanding of the interaction between these events will lead to a better understanding of how the epithelial surfaces are protected and will improve therapeutic approaches to disease in human and veterinary medicine.

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Immunization & Regulation

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Immunity & Infection

13

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Immunity and infection are inseparable. The scientific discipline of immunology was born of the study of how animals, by natural or artificial means, become immune to microbial infections and toxins. The work of Jenner concerning prophylactic immunization against smallpox was the first successful application of the observation that recovery from an infection is accompanied by acquired immunity to the infecting microorganism.

We live in a world filled with microorganisms; every facet of our existence brings us into contact with bacteria, fungi, viruses, and a diversity of other parasitic or potentially parasitic life forms. We possess a rich natural microflora on all body surfaces, within all orifices, and throughout most of the gastrointestinal tract. Even vital digestive functions are mediated partly by intestinal flora. Considering the continuous nature of our encounters with microorganisms, and notwithstanding the often mutually beneficial relationship, it is surprising that infections are not more common. However, through eons of coexistence, humans have developed sophisticated mechanisms for dealing with potential invading pathogens. Such mechanisms are the essence of **natural resistance,** which can be defined as the combined protective effects of anatomic barriers, baseline cellular phagocytosis, digestion by phagocytic cells, and effector mechanisms (such as complement), all of which are modified by nutritional status, hormonal status, and genetic makeup.

Host defenses against infection are at once local and systemic, nonspecific and specific, and humoral and cellular. It is difficult to identify any infectious agent that fails to challenge multiple host defense mechanisms; indeed, the concept of overlapping host defenses is crucial to our understanding of susceptibility to infection. Thus, "immunologic redundancy" may account for a reasonable measure of good health even in the face of an apparently significant host immune defect.

HOST DEFENSES AT BODY SURFACES

In order for an invading pathogen to produce an infection, it must somehow slip through an impressive barrier of surface defenses that operate wherever intact body tissues confront the environment. Such defenses are potent even though relatively nonspecific. They are seldom accorded the great significance they deserve.

Discharge of Microorganisms From the Body

A variety of normal functions act continually to reduce the body's bacterial burden. The mucociliary escalator of the respiratory tract brings microorganisms and foreign material to the oropharynx, where they may be coughed out or swallowed and excreted in the bowel contents. Desquamation and other forms of epithelial cell turnover at body surfaces remove large numbers of adherent microbes. Defecation results in the elimination of about 10^{12} bacteria daily, and urination eliminates microorganisms colonizing the urethral epithelium. Factors that impede diarrheal elimination of invasive microorganisms (drugs inhibiting gut motility in salmonellosis) or free passage of the urinary stream (prostatic hypertrophy) greatly enhance the risk of serious infection.

Salivation, lacrimation, and sneezing also displace potentially infective microorganisms. Patients with Sjögren's syndrome, which is characterized by severe impairment of lacrimation and salivation, are at risk of ocular and oral sepsis resulting from loss of these vital defense mechanisms.

Local Production of Chemical Antimicrobial Factors

Lysozyme (muramidase), a cationic low-molecular-weight enzyme present in tears, saliva, and nasal secretions, reduces the local concentration of susceptible bacteria by attacking the mucopeptides of their cell walls. Salivary glycolipids prevent attachment of potentially cariogenic bacteria to oral epithelial cell surfaces through a process of competitive inhibition. Similar substances present on cell surfaces apparently act as the attachment sites for bacteria. Saliva and milk contain a lactoperoxidase-SCN-HO system that possesses antibacterial activity in vitro. (Its mechanism of action is similar to that of myeloperoxidase-mediated killing in the neutrophil; see below.) Human milk also contains a lipase with potent killing ability against *Giardia lamblia* and *Entamoeba histolytica* trophozoites. Gastric acidity retards access of *Salmonella* species and *Vibrio cholerae* to the intestine. Achlorhydria and gastric resection increase susceptibility to salmonellosis, cholera, and possibly giardiasis *(G lamblia)*. Neutralization of gastric contents with bicarbonate greatly increases the susceptibility of human volunteers to cholera and shigellosis. Enveloped viruses are not, as a rule, pathogenic by the enteric route, because their lipoprotein surface membranes

are susceptible to hydrochloric acid and to enzymatic lysis. Acidity of skin and vaginal secretions retards local colonization by potential pathogens. Spermine, a polyamine present in prostatic secretions, is a potent (and highly pH-dependent) inhibitor of gram-positive microorganisms at concentrations normally encountered in semen. Seminal plasma also possesses potent bactericidal activity related to the presence of zinc.

Bacterial Interference

The normal biota of body surfaces serves an important host defense function. Not only does normal flora serve to stimulate "natural" antibody (eg, absence of a normal flora increases the susceptibility of germ-free animals to infection), but such flora also dictates to some extent the ability of potential pathogens to gain an initial foothold in the body. For example, the skin commensal *Propionibacterium acnes* appears to be capable of retarding skin colonization with *Staphylococcus aureus* and *Streptococcus pyogenes* through the production of antibacterial skin lipids. Mechanical removal of the lipids with acetone permits local multiplication of these pathogenic cocci under experimental conditions.

Anaerobic bacteria in the bowel are able to retard the local growth of *Salmonella* species through the production of fatty acids. Antibiotics that selectively eliminate the responsible anaerobes indirectly predispose to salmonellosis. Resident bowel flora may also prevent acquisition of *Shigella* species by their effect on local pH as well as by producing volatile fatty acids harmful to *Shigella*. Bile acids are excreted as glycine and taurine conjugates and are deconjugated by gut anaerobes. Deconjugated bile salts are inhibitory for a number of microorganisms, including *Bacteroides fragilis, Clostridium perfringens,* lactobacilli, and enterococci.

Viridans streptococci resident in the pharynx appear to prevent the local growth of pneumococci. *Staphylococcus epidermidis* and diphtheroids in the nasal vestibule retard colonization by *S aureus*. The mechanisms of these important microbial interactions are unclear.

Attachment to & Penetration of Epithelial Cells

Because intact skin is not easily infected, most infections in otherwise normal persons begin at mucosal surfaces. For many pathogens, the first step in initiation of infection is attachment to an epithelial surface. Ability to attach may therefore be a prime determinant of virulence. Mucous membranes are covered with a mucous gel composed of large glycoprotein molecules variably cross-linked by disulfide bridges. In the gastrointestinal tract, the gel protects the mucosa from digestion by acid or proteases. The gel is moved as a continuous layer by ciliary beating; trapped microorganisms are thereby transported from the body. The attributes of invasive microorganisms that allow them to traverse the mucous barrier are only beginning to be elucidated. Some may penetrate by innate high motility (eg, by flagella); others may be able to penetrate stress points in the mucous blanket; and still others may be vigorously attracted to the underlying epithelial cells by a process similar to chemotaxis.

Either the mucous gel or saliva may contain materials that prevent epithelial cell attachment (eg, released receptor material that competes with receptor sites on the cells themselves). Diet may also interfere with epithelial cell colonization (eg, a meat diet may contain receptors for bacterial adhesion; carbohydrate-binding proteins [lectins] from plant and animal sources, such as concanavalin A, may inhibit attachment of lactobacilli and *Escherichia coli* to mucosal cells).

A. Mechanisms of Epithelial Cell Attachment: Epithelial cell attachment is a function partially of microbial surface factors (binding sites) and partially of receptor sites on epithelial cell surfaces. There is considerable evidence that receptor sites on mammalian cells may be composed of sugars, because D-mannose, L-fucose, and D-galactose, among others, can inhibit the attachment of many bacterial species to epithelial cells. Bacteria appear to attach to sugar receptors by means of lectinlike binding sites (adhesins). Adhesins often take the form of proteinaceous, hairlike fimbriae (pili). Fimbriae appear to mediate the attachment of gonococci to mucosal epithelial surfaces and, in the urethra, may prevent the washing away of microorganisms by the force of the urinary stream. The so-called P pili of *E coli* bind to glycolipids in the cell membrane of uroepithelium and play a role in the pathogenesis of urinary tract infection. These epithelial binding sites are antigens in the P blood group system, which is found in 75% of the normal population. Fimbriae are also important in the attachment of strains of *E coli*, salmonellae, and vibrios to the intestinal epithelium in the pathogenesis of diarrhea. In a classic form of enterotoxigenic diarrhea in piglets, toxin-producing *E coli* are unable to produce diarrhea unless K-88 antigen is present on the surface of the bacteria. This antigen is distributed over the *E coli* in the form of fimbriae and mediates attachment of the bacteria to a D-mannose-resistant epithelial cell receptor site. A similar fimbria-borne antigen known as CFA (colonization factor antigen) is important in the pathogenesis of enterotoxigenic *E coli* infections in humans. Finally, attachment by *S pyogenes* to epithelial cells is mediated by lipoteichoic acid which, along with M protein, is located in surface fimbriae. However, fimbriae are not always predictive of microbial virulence; many nonpathogenic bacteria such as the common saprophytic *Neisseria* species are well fimbriated.

Other surface factors may mediate epithelial cell attachment as well. For example, fibronectin, a large glycoprotein with nonimmune opsonic function in the blood, is also found on oral epithelial cells, where it serves as an apparent attachment site for *S pyogenes*. In patients who are severely stressed, salivary protease activity may strip the fibronectin away, allowing the attachment to epithelial cells of gram-negative rods such as *Pseudomonas aeruginosa*. Thus, the presence

of fibronectin may modulate the type of flora encountered in the upper respiratory tract.

Streptococcus mutans, a mouth organism believed to be important in producing dental caries, synthesizes glucans from sucrose that promote adherence of the bacteria to each other and to dental surfaces. (*S mutans* can also attach to teeth by interacting directly with components of the enamel pellicle.) Interestingly, glucans and dextrans serve to bind endocarditis-producing streptococci to cardiac valve tissues and thus behave as virulence factors for infective endocarditis. *Mycoplasma pneumoniae* attaches to the surface of ciliated respiratory mucosal cells by a specialized tip structure. Neuraminic acid receptors may be involved in the adherence process. *Chlamydia* species lack an anatomically identifiable binding site; cell receptor specificity is mediated, at least in part, by N-acetylglucosamine.

Many viruses have surface proteins that allow attachment to specific cell membrane receptors. For example, influenza virus surface hemagglutinin attaches specifically to N-acetylneuraminic acid residues on the cell membrane; removal of these receptors prevents viral attachment. Host specificity of many viruses is determined by cell receptors, eg, whole poliovirus will not replicate in hamster kidney cells, but if infectious RNA is prepared, it circumvents the required attachment phase by direct penetration of the cell, and a single cycle of viral replication occurs.

B. Significance of Epithelial Cell Penetration: Microbial attachment is not necessarily followed by epithelial cell penetration. Infections with *M pneumoniae, Corynebacterium diphtheriae, Bordetella pertussis, V cholerae,* and enterotoxigenic *E coli* take place entirely at the epithelial surface. Other pathogens penetrate epithelial cells, where they replicate and produce the principal manifestations of disease. This is the mechanism whereby *Shigella* species infect the colon. Finally, there are pathogens which do not stop at the stage of epithelial cell invasion but proceed to systemic spread. This pattern is typical of salmonellosis.

It has become apparent that epithelial cell invasion is an important mechanism of infection for many other potential pathogens, including protozoa, spirochetes, various fungi, *Listeria monocytogenes,* and *Neisseria gonorrhoeae*. In an intraepithelial cell location, pathogens may be protected from antibodies and antibiotics and from ingestion and killing by polymorphonuclear leukocytes and mononuclear phagocytes.

Epithelial cell penetration appears to involve a process of endocytosis similar in many ways to phagocytosis. The factors that determine the "hospitality" of epithelial cells for invading pathogens are unclear. Intraepithelial microbes appear to be viable, but this is not surprising, since epithelial cells lack the comprehensive antimicrobial armamentarium of "professional" phagocytes such as polymorphonuclear leukocytes, monocytes, and macrophages. Some microorganisms, such as *Toxoplasma gondii,* appear capable of actively promoting endocytosis. The mechanism by which this is accomplished is unclear; however, toxoplasmas that are coated with antibody lose this capability even though the antibody is not directly harmful to the protozoon.

C. Host Defenses Against Attachment to Epithelial Cells: It is clearly in the interest of the host to prevent attachment of potential pathogens to epithelial cells. This may be partially accomplished by members of the normal microbial flora that attach to mucosal cells and cover up critical receptor sites. Attachment may also be retarded by local factors such as fibronectin, glycoproteins, and pH.

Antibody on mucosal surfaces plays a significant role in dictating the outcome of mucosal invasion. The antibody response at mucosal surfaces is mediated principally by secretory IgA. Secretory IgA consists of IgA antibody (synthesized by plasma cells at the local mucosal surface) covalently bound to "secretory component," a unique protein that is synthesized by mucosal epithelial cells (see Chapter 12). Mucosal immunity appears to be highly localized; elevated antibody titers in salivary secretions, for example, may not be associated with similar activity in the tears.

The mechanisms by which secretory IgA operates against microorganisms in the complex environment of secretions is not entirely clear. Secretory IgA binds antigens effectively and can neutralize viruses and bacterial enterotoxins. Mycoplasmas are inhibited in their attachment, motility, and growth. Secretory IgA cannot activate complement by either the classic or alternative pathway and appears not to be capable of promoting phagocytosis. Secretory IgA can potentiate the bacteriostatic effect of the iron-binding protein lactoferrin, which is present in secretions in abundance. As noted below, lactoferrin acts by depriving microorganisms of needed iron. Secretory IgA enhances the effects of lactoferrin by preventing bacteria from releasing their own iron-binding compounds (enterochelins, ferromyns). The clearest role for secretory IgA in bacterial disease is in the prevention of bacterial attachment to mucosal cells. Under experimental conditions, IgA can prevent the attachment of oral streptococci and *V cholerae* to mucosal surfaces. This may be why intraluminal gut antibody (coproantibody) levels are so important in protecting guinea pigs from oral challenge with *V cholerae*. Secretory IgA is believed to be important in retarding the colonization of mucosal surfaces with a variety of other microorganisms as well, including *N gonorrhoeae*. The mechanism by which secretory IgA blocks attachment of bacteria to mucosal cells is uncertain.

Not surprisingly, microorganisms have evolved countermeasures to permit their attachment to mucosal epithelial cells despite the presence of secretory IgA. *Streptococcus sanguis* and *Streptococcus mitior* (bacteria important in dental plaque formation), *Streptococcus pneumoniae, N gonorrhoeae, Neisseria meningitidis,* and *Haemophilus influenzae* have all been shown to elaborate an IgA protease in vitro that cleaves and inactivates the IgA1 subclass, yielding intact Fab and Fc fragments. The IgA2 subclass is resis-

tant to cleavage by virtue of the absence of 13 amino acid residues in the hinge region, the site at which IgA1 proteases exert their effects. Some strains of *Bacteroides asaccharolyticus, Bacteroides melaninogenicus,* and *Capnocytophaga* are capable of cleaving not only IgA1 but also IgA2 and IgG. These bacteria are important etiologic agents in human periodontal disease. Immunoglobulin protease activity has been detected in human oropharyngeal secretions, dental plaque, and vaginal secretions. It is possible that these proteases play an important role in the interaction of bacteria and epithelial cells.

SYSTEMIC IMMUNITY TO INFECTION

Cellular Systems of Systemic Immunity

Once microorganisms have breached local defense mechanisms, a number of tightly integrated immunologic events are called into play that are predominantly related to the activity of 2 types of phagocytic cells: polymorphonuclear leukocytes and mononuclear phagocytes. These cells have been termed "professional phagocytes" because their membranes possess specialized receptors for the Fc portion of IgG molecules (IgG1 and IgG3 subclasses) and for activated C3. These receptors augment the process of phagocytosis by assisting in the ingestion of microorganisms with IgG or activated C3 on their surfaces. Nonprofessional or facultative phagocytes, in contrast, include endothelial cells, epithelial cells, fibroblasts, and other cells which will ingest microorganisms under specified conditions but which do not possess specialized membrane receptors for IgG or C3.

Mononuclear phagocytes are the only phagocytic cells of nonvertebrates and subserve a digestive function in at least some of them. Granulocytes are found in animals possessing circulatory systems. Why the need arose for 2 different types of phagocytic cells whose capabilities overlap in many respects is still unknown. However, many consider the polymorphonuclear neutrophil (PMN), by virtue of its peculiarly segmented nucleus, to be especially well designed to traverse tight spaces.

A. Polymorphonuclear Neutrophil Leukocytes: These cells are concerned principally with the destruction of microorganisms that rely upon the evasion of phagocytosis for survival. Once ingested, such microorganisms generally perish. Microorganisms of this type are considered **extracellular pathogens;** their prototype is the pneumococcus.

B. Mononuclear Phagocytes: These cells are concerned principally with the control of microorganisms which are able to survive intracellular residence and against which neutrophils are ineffective. The principal effector cells are monocytes and macrophages. Monocytes are immature circulating forms of mature tissue macrophages. Monocytes may serve as a backup system to neutrophils in acute infections, but they phagocytize less efficiently and lack many of the potent bactericidal systems of the neutrophil. Macrophages are much more important in chronic infections. Sensitized lymphocytes may augment the bactericidal activities of macrophages through direct cell-to-cell contact or by the intervention of soluble mediators (monokines, lymphokines, interleukins, gamma interferon). Conversely, macrophages may process ingested or adsorbed microbial antigens preparatory to the sensitization of lymphocytes. Intracellular pathogens such as *Mycobacterium leprae, Mycobacterium tuberculosis, L monocytogenes, Salmonella typhi,* and some viruses and protozoa appear to be under control of lymphocytes and macrophages. (See also Chapter 9.)

Humoral Systems of Systemic Immunity

The role of humoral factors in systemic infection is largely related to augmentation of phagocytic function through the processes of chemotaxis and opsonization. In many respects, the humoral immune system provides specificity to the phagocytic system. However, some mechanisms of antimicrobial activity are mediated solely by humoral factors.

A. Complement-Mediated Bacteriolysis: In the presence of specific antibody and an intact classic complement pathway, many gram-negative bacteria can be directly lysed by serum. Among these are *N gonorrhoeae, N meningitidis, H influenzae,* and strains of *Salmonella, Shigella,* and *Vibrio.* Endotoxin in the cell membrane of gram-negative bacteria, unless blocked by other membrane constituents, may also directly activate the alternative complement pathway, with the result that bacteriolysis occurs in the absence of antibody. Thus, the alternative complement pathway may serve as an important first line of defense in the nonimmune host—a role that it also plays in regard to phagocytosis (see below). In order for bacteriolysis to occur, activation of the entire terminal attack sequence of the complement cascade is ordinarily required. The importance of terminal complement components is illustrated by the fact that patients with deficiencies of C6, C7, or C8 may suffer repeated episodes of gonococcal or meningococcal sepsis.

Unlike the situation described above, specific antibody and both the classic and alternative complement pathways subserve a predominantly opsonic function for gram-positive bacteria and fungi.

Bacterial variants that have lost cell wall material (L forms) are susceptible to complement-mediated lysis even when the gram-positive and gram-negative microorganisms from which they are derived are resistant to the effects of antibody and complement.

B. Viral Neutralization: Humoral antibodies constitute one of the more important mechanisms of host resistance to viral infections. (See Special Aspects of Viral Immunity, below.)

C. Beta Lysin: Beta lysin is a highly reactive heat-stable cationic protein which is bactericidal for gram-positive microorganisms (except streptococci). Its release from platelets during coagulation results in

serum levels which are far higher than plasma levels. Beta lysin acts at the cell membrane of gram-positive bacteria to produce a nonenzymatic destructive effect similar to that of histones. Gram-negative microorganisms are resistant to its effects except under experimental conditions or in the presence of antibody, complement, and lysozyme.

D. Lysozyme: Lysozyme is a basic protein that originates from phagocytic cells and is present in serum in a concentration of 1–2 μg/mL. It is actively secreted by monocytes and macrophages. Its mechanism of action is discussed later.

Microbial Agglutination & Bloodstream Clearance

Bacteria that gain access to the circulation are generally cleared from the blood by the fixed tissue macrophages of the mononuclear phagocyte system—especially the Kupffer cells of the liver. Features of opsonization and phagocytosis are presumably the same as for other mononuclear phagocytes (see below). However, there is evidence that the agglutination of microorganisms by serum factors, perhaps on a nonspecific basis, serves to augment the clearance of microorganisms from the bloodstream as well.

THE MONONUCLEAR PHAGOCYTE SYSTEM & ITS FUNCTION

The mononuclear phagocyte system has its origin in the bone marrow monoblast and promonocyte. Only the intermediate stage cell (the monocyte) is ordinarily encountered in the circulation. The ratio of circulating to marginated monocytes in humans is approximately 1:3. Monocytes circulate with a half-life of 8½ hours, leaving the circulation randomly (ie, unrelated to age). There is a daily monocyte turnover of approximately 7×10^6 cells per hour per kg body weight (see Chapter 9).

Unlike neutrophils, monocytes do not die when they leave the circulation but mature to macrophages (histiocytes) in the tissues: alveolar macrophages (lung), Kupffer cells (liver), and macrophages of spleen sinusoids, lymph nodes, peritoneum, and other areas. There is good evidence that macrophages in the lung and liver may proliferate locally as well. Macrophage maturation is accompanied by an increase in cell size and in numbers of cytoplasmic organelles including mitochondria and lysosomes (containing hydrolytic enzymes) as well as other morphologic, biochemical, and functional changes. These changes vary from tissue to tissue according to the state of the host (normal, infected, or otherwise stimulated). The synthetic activities of macrophages can also be stimulated in culture, a good example being the increase in lysosomal hydrolases after exposure in vitro to foreign serum. Functional maturity of these cells is shown by increasing phagocytic capability, increased numbers of Fc receptors for IgG on the cell surface, and increased responsiveness to lymphocyte activation. Mature macrophages in unique environments may achieve distinctive cellular physiology. For example, alveolar macrophages, like monocytes (see below), depend predominantly on aerobic metabolism for their energy supply, whereas peritoneal macrophages depend primarily on glycolytic metabolism. In addition, alveolar macrophages contain large amounts of lysozyme, which is at least partially endocytized from respiratory secretions.

Chemotaxis

Humoral mediators of mononuclear phagocyte chemotaxis are less well understood than are neutrophils. However, monocyte chemotaxis occurs in response to synthetic N-formylated oligopeptides, and C5a provides chemotactic activity from the serum. Recruitment of mononuclear phagocytes is effected to a major degree by chemotactic materials released from sensitized T lymphocytes. Additional lymphocyte-derived substances, such as migration inhibitory factor, encourage the accumulation of chemotactically attracted phagocytes in inflammatory foci.

Opsonization

A. General Aspects of Opsonization: The function of serum opsonins (Gk *opsonein* to prepare food for) is to react with microorganisms and make them more susceptible to ingestion by phagocytes. The virulence of many pathogens relates in part to their ability to evade phagocytosis by virtue of certain surface antigens. Microorganisms in which antiphagocytic surface factors are of importance include *Streptococcus pneumoniae*, group B streptococci, *Klebsiella pneumoniae*, *H influenzae*, *N meningitidis*, *B fragilis*, and some strains of *P aeruginosa* (capsular polysaccharides); *Bacillus anthracis* (capsular polypeptide); *N gonorrhoeae* (pili composed of protein; other poorly characterized surface factors); *S pyogenes* (capsular hyaluronic acid and M protein, the latter binding fibrinogen and impeding access of complement protein to cell wall structures); and *S aureus* (protein A, which has the capacity to bind to the Fc portion of IgG, thereby competing with phagocytes for the Fc sites of opsonins).

Van Oss has postulated that nonvirulent bacteria possess relatively hydrophobic surfaces that favor phagocytosis, whereas virulent (especially encapsulated) microorganisms are characterized by hydrophilic surface factors that retard phagocytosis. According to this view, it is the purpose of opsonization to increase hydrophobicity, thereby reducing the charge repulsion between microorganism and neutrophil, both of which are negatively charged.

Opsonization of bacteria may occur by at least one of 3 mechanisms, as noted below. However, no simple scheme can summarize the opsonic requirements of any single genus or species of microorganism.

First, specific antibody alone (subclasses IgG1 and IgG3) may act as an opsonin as shown in Fig 13–1. This mechanism has been explored most thoroughly in studies employing pneumococci under conditions of

abundant antibody. Here, anticapsular antibody combines with the surface polysaccharide antigens of the pneumococci through antibody combining sites located on the Fab portion of the immunoglobulin molecule. The Fc portion of the molecule, which is critical to its function as an opsonin, is then free to attach to Fc receptor sites on the surface of phagocytes, thus completing a bridge between bacteria and phagocytic cell.

Second, specific antibody acting in concert with complement via the classic C1, C4, C2 pathway may promote microbial opsonization. Here, a quantity of IgM or IgG apparently insufficient to opsonize on its own may react with bacteria and activate sequentially the hemolytic complement sequence. Receptor sites for 2 fragments (C3b and C3bi) of the third component of complement are present on the surface of phagocytes (Fig 13–1). The activated C3 on the bacterial surface apparently serves as a bridge between bacteria and phagocyte, but it is insufficient in itself to promote ingestion. For phagocytosis to occur, either large numbers of C3 receptors must be engaged, or (for macrophages) there must be participation by a lymphokine that "activates" the Fc receptor—apparently by allowing these otherwise fixed receptors to migrate and redistribute within the cell membrane.

Third, opsonization can be nonspecific, via the alternative complement pathway. Although antibody is absolutely required for opsonic activity mediated by the classic complement pathway, the alternative pathway does not require antigen-antibody interaction. Instead, this pathway is activated directly by bacterial or fungal polysaccharides, resulting in fixation of C3, the crucial opsonic factor, to the surface of the microorganism. Ingestion by phagocytes is therefore mediated by the cellular receptor for activated C3.

Normal 7S immunoglobulin isolated from nonimmune animals has been shown to participate functionally in this system (at least in the case of pneumococci). The immunoglobulin does not appear directed toward the capsule, and its site of binding on the pneumococcus is unknown.

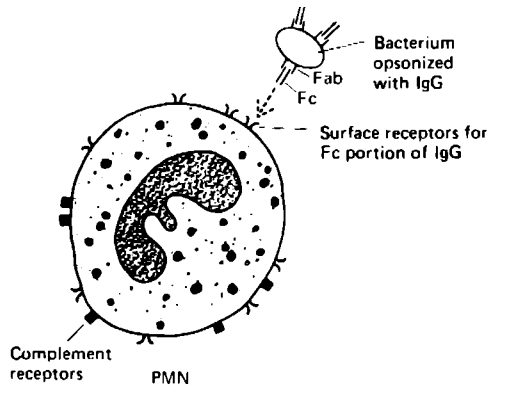

Figure 13–1. Schematic representation of receptors on the surface of a neutrophil (PMN) that interact with complement components and with the Fc portion of IgG molecules. (Reproduced, with permission, from Cline MJ: *The White Cell*. Harvard Univ Press, 1975.)

Since the alternative complement pathway is present in nonimmune animals and is not dependent upon the presence of anticapsular antibody for its action, it has been considered to play an important role in the early and critical preimmune stages of infection prior to the production of specific antibody. This is also the stage at which surface phagocytosis is considered most important (see below). The precise role of the alternative complement pathway in human infection is not clear, but it has been suggested that the abnormal susceptibility of patients with sickle cell anemia to fulminating pneumococcal infection may be related to their demonstrably low levels of heat-labile pneumococcal opsonins. Such patients may fail to utilize fully the alternative pathway of complement activation, perhaps because some of the necessary components are synthesized in the spleen. Although levels of factor B and properdin are normal, there is a deficiency of C3 proactivator convertase (C3PA) activity. Patients with sickle cell anemia undergo early autosplenectomy as a result of repeated infarcts.

B. Other Factors Promoting Phagocytosis: There are 3 other factors that may promote phagocytosis of microorganisms in association with the above systems.

1. Surface phagocytosis—As noted, many microbial pathogens may possess antiphagocytic surface components (such as pneumococcal capsular polysaccharide) that protect them from phagocytosis in the absence of specific antibody. Yet there are often no homologous antibodies in the serum before the fifth or sixth day of illness. Survival of a patient during the preantibody stage of infection may depend not only upon the alternative complement pathway but also upon surface phagocytosis. Here, encapsulated bacteria are trapped between leukocytes themselves, between leukocytes and tissue surfaces, or along with leukocytes in the interstices of fibrin clots. Surface phagocytosis may occur with mononuclear phagocytes as well as neutrophils. Heavily encapsulated type 3 pneumococci may resist surface phagocytosis for some time. Surface phagocytosis is much less efficient in areas where leukocytes are not tightly packed (pleural, pericardial, and joint fluids; cerebrospinal fluid).

2. Natural antibody—Antibodies present in the serum in the absence of apparent specific antigen contact are referred to as natural antibodies. They probably reflect contact with microorganisms possessing related antigens. For example, more than 80% of persons over the age of 1 year have antibody to type 7 pneumococci, although the carrier rate of this microorganism is only about 1%. The antibody appears following exposure to certain viridans streptococci that possess a cross-reacting surface antigen.

Presumably, natural antibodies may participate with the heat-labile opsonin system in preventing in-

fection with microorganisms that do not possess surface factors posing a serious challenge to phagocytosis.

3. Tuftsin—Leukokinin, a γ-globulin moiety that coats the polymorphonuclear neutrophil (PMN), is capable of stimulating phagocytosis by neutrophils under experimental conditions. The biologic activity of leukokinin rests in a single peptide, tuftsin—so called because it was discovered at Tufts University. The peptide (Thr-Lys-Pro-Arg) is apparently produced in the spleen. It has also been prepared synthetically. A membrane enzyme of the neutrophil appears capable of splitting tuftsin from leukokinin. Splenectomy results in a severe deficiency of tuftsin and may be one of several reasons why splenectomized patients are more susceptible to certain infections.

C. Special Aspects of Opsonization for Mononuclear Phagocytes: The membranes of human monocytes and macrophages contain receptors for IgG (subclasses IgG1 and IgG3) and C3. Presumably, therefore, the process of opsonization is similar to that which occurs with neutrophils. *Mycoplasma* species have the interesting ability (in vitro) to attach to macrophages in the absence of antibody or complement. When so attached, they retard ameboid movement of the phagocytic cell. Upon addition of specific antibody, mycoplasmas are phagocytized and killed. Such experimental observations permit dissection of the attachment and ingestion phases of mononuclear phagocyte function, but their practical significance is uncertain.

Ingestion

Monocytes ingest bacteria more slowly than neutrophils, kill them less efficiently, and utilize predominantly oxygen-dependent metabolic pathways (oxidative phosphorylation) to accomplish phagocytosis. Nevertheless, a respiratory burst clearly accompanies monocyte-microbial encounters, with generation of H_2O_2, $O_2^{-}\cdot$, and $OH\cdot$ and with chemiluminescence.

Killing

Monocytes possess 2 lysosomal populations. The first appears early in monocyte maturation and contains myeloperoxidase, arylsulfatase, and acid phosphatase. The contents of the second, later-appearing lysosomes are unknown. Monocytes do not possess the bactericidal cationic proteins of neutrophils; lactoferrin is absent. However, the MPO-H_2O_2-halide system is apparently operative, and monocytes from patients with chronic granulomatous disease have impaired microbicidal activity.

As monocytes mature to macrophages in the tissues, additional lysosomal structures develop on a continuing basis reflecting both the prolonged life span of these phagocytic cells and their ability to synthesize new membrane and membrane receptors. Preexisting primary lysosomes may fuse with phagocytic vacuoles or pinocytotic vesicles to produce secondary lysosomes. At least in vitro, their formation and contents are closely related to the extracellular milieu. There is a definite relationship between endocytic activity and the formation of lysosomes; as macrophages mature, there is a progressive rise in lysosomes and their hydrolytic enzyme content.

The mechanisms by which macrophages kill microorganisms are not understood. Myeloperoxidase is not found beyond the monocyte stage of macrophage development, and there is a progressive decline in the ability of maturing macrophages to generate $O_2^{-}\cdot$ and H_2O_2. Enzymes that are found in mononuclear phagocytes (including acid phosphatase, β-glucuronidase, lipase, lysozyme, hyaluronidase, and others) appear to serve a digestive rather than a microbicidal function. In the presence of oxygen and clofazimine, a phenazine dye used in the treatment of leprosy, H_2O_2 generation in the human macrophage is stimulated and killing is enhanced. Possibly, therefore, macrophages employ an H_2O_2-generating system in microbial killing. Catalase, which is present in macrophages, may substitute for myeloperoxidase, which is not present, and may thus catalyze the oxidation of substrates in the presence of H_2O_2. Under in vitro conditions, catalase can substitute for myeloperoxidase in the myeloperoxidase-H_2O_2-halide microbicidal system. Lipid peroxidation, which occurs in alveolar macrophages and monocytes, may be another mechanism potentiating the antimicrobial action of H_2O_2 because malonyldialdehyde, a catabolite of lipid peroxides, has antibacterial activity.

Secretory Products of Mononuclear Phagocytes

These cells secrete or shed into their environment a variety of biologic products that may be important in terms of mediating the immune response. For example, lysosomal acid hydrolases are produced by mouse peritoneal macrophages even in the absence of a phagocytic stimulus. Lysozyme is secreted in large amounts from a variety of mononuclear phagocytes in the presence or absence of phagocytic stimuli; the source does not seem to be lysosomes. Neutral proteases have been identified as secretory products of macrophages. Other products include prostaglandins, complement and properdin components, fibroblast growth-regulatory agents, regulators of angiogenesis, bone marrow stem cell stimulators, interferon, and blood coagulation-regulating substances.

Fate of Intracellular Microorganisms

Depending upon their ability to survive intracellular conditions, phagocytized microorganisms may be killed and digested, killed and poorly degraded, or not killed and merely sequestered within the cells. Some microorganisms have apparently developed mechanisms for ensuring their intracellular survival and replication in macrophages. *M tuberculosis* is admirably equipped in this regard. In the absence of immune serum, tubercle bacilli prevent the fusion of lysosomes with phagosomes, apparently through the mediation of a markedly anionic trehalose glycolipid. In the presence of immune serum phagolysosomal fu-

sion occurs, but *M tuberculosis* is resistant to the discharged lysosomal contents. The interaction of *Legionella pneumophila* with human mononuclear phagocytes appears to be unique. During the process of ingestion, a monocyte pseudopod coils around the microorganism to facilitate cell entry. Immediately after ingestion, cellular organelles fail to appear about the phagosome. Subsequently, the bacteria are noted to be multiplying in a ribosome-studded vacuole. It has been noted that these vacuoles have some features in common with those of other intracellular organisms that inhibit phagolysosomal fusion.

Mycobacterium lepraemurium and *M leprae* are also resistant to phagolysosomal contents, and the latter organism is also apparently capable of escaping into the cytoplasm of phagocytes, where it is not recognized as a foreign invader. Typhus-producing rickettsial species also escape from phagolysosomes and multiply freely in the macrophage cytoplasm in the absence of specific antibody. In contrast, antibody-coated rickettsiae are unable to escape the phagolysosome. Some obligate intracellular parasites gain entry to the cytoplasm directly instead of by rupture of the phagosome (eg, *T gondii, Chlamydia* species). Entry is apparently actively mediated by the microorganisms. In the presence of specific antibody, this parasite-mediated endocytosis is blocked while true phagocytosis is facilitated.

Cell-Mediated Immunity (Lymphocyte-Macrophage Interaction)

Acquired resistance to a broad range of intracellular parasites has its origin in a cell-mediated immune response involving both macrophages and lymphocytes. During the induction of immunity, macrophages probably facilitate the engagement of antigen-sensitive T lymphocytes. Antigen-activated T cells, the specific mediators of cellular resistance to infection, are generated in regionally stimulated lymphoid tissue and then released into the general circulation.

Interactions between mononuclear phagocytes and sensitized lymphocytes occur during expression of cellular resistance to infection. Sensitized lymphocytes produce soluble factors (lymphokines), one of which, MIF, encourages circulating blood monocytes to localize at sites of microbial invasion. In addition, lymphocytes specifically committed to microbial antigens can be stimulated (at least in vitro) to release products that enhance (activate) the endocytic and microbicidal capacity of macrophages (Fig 13–2). The mechanisms by which lymphokines (probably gamma interferon) might augment macrophage killing are unclear. Indeed, whether such factors actually function in vivo requires further documentation. The principal role of sensitized lymphocytes may be to encourage accumulation of abundant mononuclear phagocytes at foci of microbial invasion.

A full description of the "activated macrophage" is not yet at hand. Activated macrophages spread out more extensively on glass, exhibit more Fc and Fab receptors, and are more actively phagocytic than unstimulated cells. In addition, there is increased oxidation of glucose, increased lysosomal hydrolase and ectoenzyme activity, and increased antimicrobial and antitumor activity. The reason for the enhanced antimicrobial activity is not clear, ie, whether it is related to increased metabolism or reflects merely increased phagocytosis. There is recent evidence that antimicrobial and antitumor functions can be dissociated, with the latter requiring a higher degree of macrophage activation than the former.

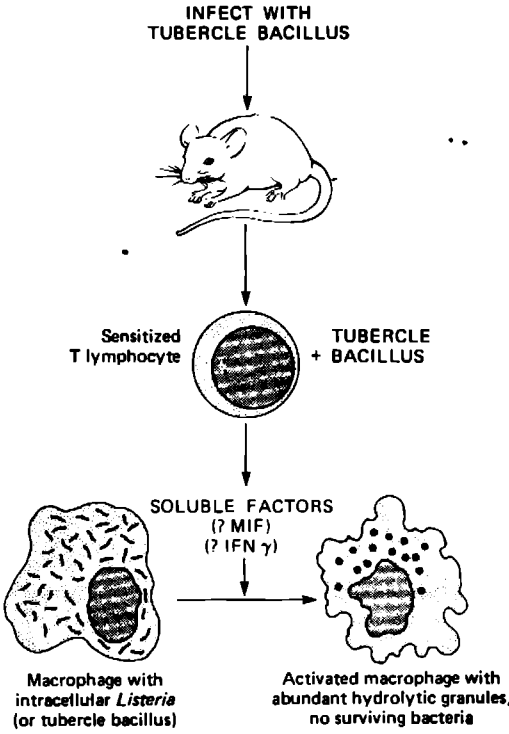

Figure 13–2. Macrophage killing of intracellular bacteria triggered by specific cell-mediated immunity reaction. (The final stage shown is probable but still hypothetical.) (Redrawn and reproduced, with permission, from Roitt IM: *Essential Immunology*, 2nd ed. Blackwell, 1974.)

Although cell-mediated immunity may be induced specifically through the action of sensitized lymphocytes, it is expressed nonspecifically in the sense that macrophages, once stimulated to a state of enhanced microbicidal activity, will perform this function nonspecifically. Thus, animals infected with *T gondii* will more readily limit infection with *L monocytogenes* and other intracellular parasites through the presence of activated macrophages. The practical significance of this observation is unclear, however, since experimental animals appear to eliminate the homologous sensitizing microorganism much more efficiently than the heterologous challenge microorganism.

The importance of lymphocytes in immunity to in-

tracellular infection is well demonstrated by the phenomenon of adoptive immunity, wherein lymphocytes transferred from an animal with immunity to a given infection (such as tuberculosis) confer immunity to the same pathogen upon a normal animal (Fig 13–3). The animals must be closely related genetically to demonstrate this phenomenon; otherwise, a GVH reaction might ensue. The use of transfer factor (an immunologically active dialyzable lymphocyte extract) in humans is based upon the principles of adoptive immunity. Since whole lymphocytes are not transferred between individuals, a GVH response is not a problem.

There is a close relationship between cell-mediated immunity and delayed hypersensitivity; skin test reactivity to a given pathogen is generally transferred along with adoptive immunity to that pathogen. These processes are not inseparable, however. Thus, RNA extracted from *M tuberculosis* may transfer immunity but not skin test reactivity. Conversely, mycobacterial lipids may be more important in the delayed hypersensitivity skin test response than in immunity.

SPECIAL ASPECTS OF VIRAL IMMUNITY

Viral Spread & Replication

Because viruses are obligate intracellular parasites, the mechanism of immune restriction of virus replication differs significantly from that of bacteria. Although extracellular viruses may be removed by the same humoral and cellular mechanisms that remove bacteria, the intracellular replicative steps of viruses and virus-infected cells (that have developed virus-specified antigens on their surface) are also major targets for the host's immune response. The mechanism by which viruses are transmitted also differs considerably from that of bacteria.

A. At the Cellular Level: At the cellular level, Notkins has defined 3 types of viral transmission (Fig 13–4).

1. Extracellular (type I) spread—Infectious virions are released from the cell to spread in the extracellular milieu. Many types of viruses (eg, influenza, adenoviruses) spread primarily by this route, and most spread by this mechanism at least some of the time.

2. Intercellular (type II) spread—Virions spread from cell to cell through desmosomes of intercellular bridges (cell fusion) without contact with the extracellular milieu. Members of the herpesvirus group (especially cytomegalovirus, EB virus, and varicella-zoster virus) are transmitted primarily by this means.

3. Nuclear (vertical; type III) spread—The viral genome is latent or integrated into the host genome and is passed from parent cell to progeny during meiosis. Phenotypic evidence of viral presence may be striking (many virus-specific antigens on the cell surface) or absent, and the stability of integration is variable. Retroviruses are a classic example of this mechanism.

B. In the Host: Within an animal host, 3 general types of viral spread are recognized:

1. Local—Viral infection is largely confined to a mucosal surface or organ—eg, rhinoviruses (respiratory epithelium), rotaviruses (gastrointestinal epithelium).

2. Primary hematogenous—Virus is inoculated directly into the bloodstream, with subsequent organ dissemination. The best examples are arboviruses and hepatitis B virus.

3. Secondary hematogenous—Initial virus infection and replication occur on a mucosal surface; bloodstream invasion occurs afterward by hematoge-

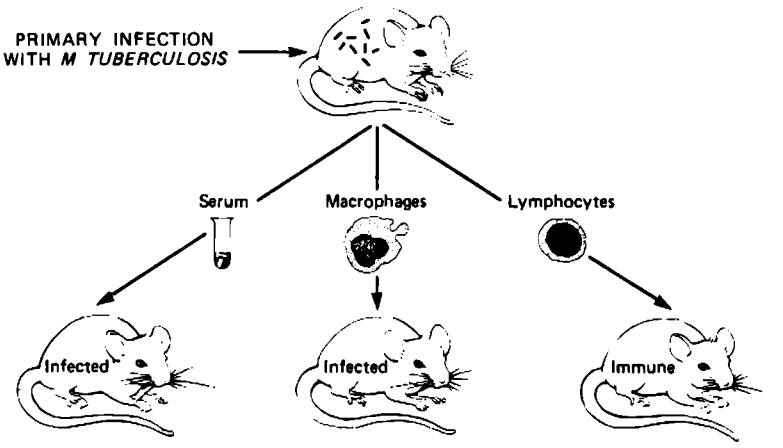

Figure 13–3. Transfer of specific and nonspecific immunity by lymphocytes from an immune animal. The recipient of the lymphocytes resisted simultaneous challenge with *M tuberculosis* combined with *Listeria* organisms. The recipients were not immune to *Listeria* given alone. Serum or macrophages did not transfer immunity. (After Mackaness.)

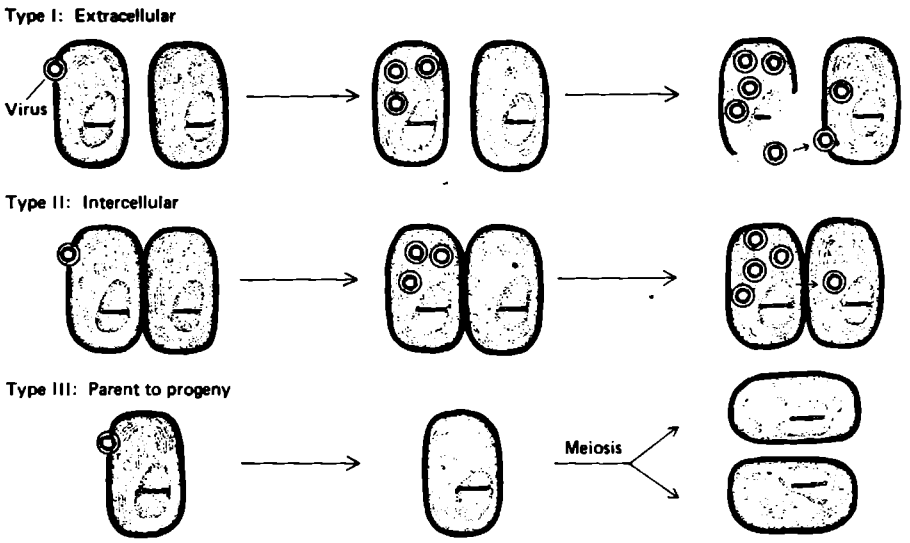

Figure 13–4. Three routes of viral spread are recognized. In type I spread, the infected cell is lysed and the progeny virus particles spread extracellularly to near and distant uninfected cells. Other viruses induce intercellular connections, allowing them to spread from cell to cell without leaving the cytoplasm (type II spread). In type III spread, viral genetic information is incorporated into the cell genome and passes to progeny during meiosis. (Reproduced, with permission, from Notkins AL: Viral infections: Mechanisms of immunologic defense and injury. *Hosp Pract* [Sept] 1974;9:66.)

nous dissemination to target organs. The initial mucosal phase is often relatively asymptomatic. Examples include common viral exanthems, poliomyelitis, and mumps.

4. Neural—Certain viruses that are inoculated at peripheral sites spread via the nervous system. Examples include rabies and herpes simplex.

Virus-Host Cell Interactions

Infection of cells by extracellular virus (type I spread) is invariably initiated by binding of virus to the surface of the host cell. In all instances studied, this binding was mediated by interaction between ligands on the virus and specific cell receptors; however, the nature of the receptor and the ligand is known in only a few instances (eg, the AIDS retrovirus glycoprotein that binds to the CD4 antigen of lymphocytes). Virus ligand-cell receptor interactions may have a profound effect on the pathogenesis of virus infections. For example, the neurovirulence of reoviruses is mediated exclusively by the type 3 hemagglutinin protein, and recombinants of reoviruses types 1 and 2 that contain the type 3 hemagglutinin become neurovirulent. Alteration of virus ligands (eg, by mutation or by selection through growth in neutralizing monoclonal antibodies) also can alter pathogenesis. For example, variants of virulent rabies virus selected because they were resistant to neutralization by a monoclonal antibody were avirulent; amino acid sequence data showed that this change was due to a single amino acid substitution. Conversely, modulation of virus receptors on cells may also alter pathogenesis. For example, resting lymphocytes lack receptors for encephalomyocarditis virus and cannot be infected; exposure of lymphocytes to mitogens induces virus receptors and confers susceptibility to infection. The expression of virus receptors is also commonly altered during cell differentiation. Alteration of cell receptors by the virus infection has been hypothesized as one mechanism whereby autoimmune disease might be triggered. Likewise, infection of a cell with one virus could induce receptors for a second virus. Alternatively, viruses bound to IgG antibody or complement may exhibit increased infectivity for cells with surface Fc or complement receptors.

Properties of Viruses That Allow Them to Escape Immunologic Defense Mechanisms

A. Poor or Absent Cellular or Humoral Immune Response to Viral Antigens: As most viruses are good antigens, the immune response is usually vigorous. However, viruslike obligate intracellular parasites such as the etiologic agent of scrapie do not induce a detectable immune response. In addition, viruses may be immunosuppressive (see below).

B. Type II or III (Intracellular) Spread: Although this mechanism of transmission limits exposure of extracellular virus to humoral or cellular host defense mechanisms, the virus-infected cells (with virus-specified surface neoantigens) are themselves susceptible to immunologic attack.

C. Serologic Plasticity (Influenza Virus) or

Multiplicity (Enteroviruses, Rhinoviruses): Some viruses, best exemplified by influenza, are capable of rapidly changing their surface antigenic structure by mutation or recombination (or both), allowing sequential infection of human and animal hosts. Thus, a single person may be infected with influenza virus 5 or 10 times or more in a lifetime. These antigenic changes are responsible in part for repeated influenza epidemics. This is in contrast to a structurally related virus, measles, that exhibits marked serologic stability and elicits lifelong immunity. Other viruses have evolved a large number of serologic variants with little cross-reactivity among them. The best examples are the rhinoviruses (≥ 82 serotypes) and the enteroviruses (≥ 66 serotypes). Sequential infections with these agents are common.

D. Nonneutralizing Virus-Antibody Complexes (eg, Hepatitis B, Lactic Dehydrogenase Virus): This phenomenon probably occurs to some extent with all viruses (as the virus-antibody reaction is reversible), but with some viruses the reaction with antibody seems to have very little effect on infectivity. These antibodies may produce immune complex disease (see Chapter 11).

E. Antibody Modulation of Virus Antigens on Cells: Virus-infected cells often have viral antigens on their surface, and the expression of these antigens may be altered by antiviral antibody. For example, exposure of measles virus-infected cells to antibody results in redistribution of measles virus antigens on the cell envelope, and these "capped" cells are resistant to killing by antiviral antibody or lymphocytes. These "capped" cells may permit persistence of virus in the face of a vigorous host immune response.

F. Camouflage: Virus proteins on the surface of virus-infected cells may bind normal host materials, obscuring the virus antigens and deceiving the immune system. A good example of this is the Fc receptor activity of certain herpesvirus glycoproteins that causes the surface of the infected cell to be covered by host immunoglobulins.

G. Immunosuppression: Virus infection may suppress the host's immune response. The mechanisms are not well worked out, but a direct effect on immunocytes (eg, cytomegalovirus; retroviruses) or production of immunosuppressive viral proteins (retroviruses) have both been described.

H. Latency: Viruses may become latent (dormant) within infected cells by a number of molecular mechanisms. Viral antigens are scarce or absent on the cell surface.

Immunologic Reactions Involving Viruses

A variety of immunologic reactions involving viruses have been shown to occur in vitro; evidence that they are operative in vivo and are important in host defense against infection is less complete.

A. Humoral Defense Mechanisms: Antibodies constitute one of the important mechanisms of host resistance to viral infection. They appear to be more important in preventing infection or dissemination than limiting an infection that has already begun. Virus-antibody reactions that have been defined in vitro include neutralization (both with and without complement), complement-facilitated lysis of infected cells (Fig 13–5), opsonization, and enzyme inhibition.

1. Complement-independent neutralization—Antibodies of the G, M, and A classes have been shown to neutralize the infectivity of virtually all known viruses; this is the most important virus-antibody interaction. The reaction is highly specific, as the antibody is synthesized in response to viral antigens, but it is only effective against extracellular virus (type I spread). With viruses that disseminate intracellularly (type II or type III spread), neutralizing antibody alone will be ineffective once infection is established.

In every instance that has been studied, the mechanism of viral neutralization involves combination of antibodies with the virus coat proteins. In most cases the antibodies prevent cellular adsorption and penetration of the virus, but in some instances antibody coating of extracellular virus may interfere with subsequent intracellular events (eg, uncoating) or may physically aggregate virus particles. The exact mechanism of neutralization is unclear but presumably involves a change in the steric conformation of the virus surface or actual covering of receptor sites, either of which prevents the virus from gaining entry to the cells. In some instances, a single antibody molecule can neutralize a virion with many repeated sequences of surface antigen, suggesting that conformational changes may be more important than simple interference. Neutralization of this sort is reversible in that the virus-antibody complexes may be dissociated to yield infectious virions.

IgA often plays a role in viral infections which begin on or are confined to mucosal surfaces. In the case of rhinovirus infections and perhaps parainfluenza and respiratory syncytial virus infections of adults, where viral replication may be confined to respiratory epithelium, resistance to infection is determined by surface IgA; serum IgG neutralizing antibody has much less protective value. For viral infections which begin on a mucosal surface and then disseminate by hematogenous spread (eg, poliomyelitis, measles, rubella), local antibody may completely prevent infection. However, disease can also be prevented by serum antibody even though viral replication still may occur on the mucosal surface. Thus, persons immunized parenterally with inactivated poliovaccine possess serum antibody to poliovirus but not local colonic or oropharyngeal secretory antibody. Although still susceptible to mucosal infection with poliovirus, they are resistant to disease. During such an infection, virus will be found in oropharyngeal secretions and stool but not in blood or neural tissue.

2. Complement-facilitated neutralization—There is good evidence that complement plays an important role in neutralization of extracellular virus. Complement alone can neutralize some enveloped viruses by direct lysis (eg, retroviruses) or by occlud-

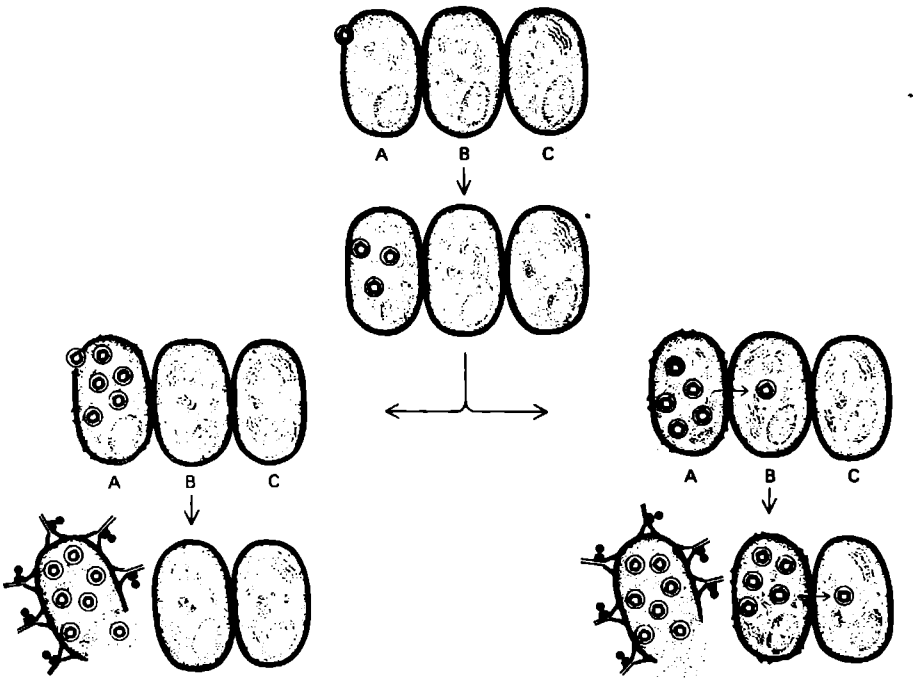

Figure 13–5. Virus-infected cell can be destroyed by antiviral antibody and complement, but whether this halts type II spread depends in part on the speed of transmission. If, for example, infected cell A is immunologically destroyed before the virus is transmitted to cell B, the infection will be stopped *(left)*. If, on the other hand, the virus transmission to cell B occurs before cell A can be destroyed immunologically, the infection will progress *(right)*. (Reproduced, with permission, from Notkins AL: Viral infections: Mechanisms of immunologic defense and injury. *Hosp Pract* [Sept] 1974;9:70.)

ing surface structures required for attachment of virus to host cells (eg, herpesviruses). However, it is primarily important as an adjunct to neutralization by specific antibody, in which it may enhance antibody-mediated steric changes or aggregation, thus preventing adsorption of virus to cells, or may directly lyse enveloped viruses. Complement coating of virions may also facilitate binding to and ingestion by phagocytic cells (see below). The importance of complement-facilitated neutralization in determining in vivo resistance to viral infection has not been determined, although as noted below, complement-deficient animals are generally more susceptible to virus infection than normal animals. IgA (in contrast to IgG and IgM) does not fix complement well, and this class of antibody has not been shown to function in complement-dependent neutralization reactions.

3. Lysis of virus-infected cells—Infected cells that have virus-specified antigens on their surfaces are susceptible to cytolysis by antibody and complement. The elegant studies of Sissons, Oldstone, and collaborators have shown that virus-infected cells are lysed primarily through the alternative complement pathway, in contrast to nonnucleated cells such as erythrocytes. Some (perhaps many) types of viruses can activate the alternative complement pathway directly; however, this activation is rarely sufficient to induce cytolysis unless deposition of C3b on the cell surface is amplified by antibody. Relatively large amounts of antibody molecules per cell are required, and the Fc portion of the molecule is not essential for the reaction. Although the classic complement pathway may be activated on the surface of virus-infected, antibody-coated cells, it does not seem to be important for cytolysis.

As yet, there is no evidence to directly support a role for antibody-dependent complement-mediated cytolysis in host resistance to virus infection. On the other hand, virus infection of complement-deficient or complement-depleted mice generally results in more severe infection than in normal mice, favoring an important role for complement in resistance to virus infection.

4. Opsonization—Coating of extracellular viruses by IgG antibody, complement, or both, may facilitate phagocytosis and intracellular destruction by macrophages or polymorphonuclear leukocytes. On the other hand, cell penetration and replication of some viruses within macrophages is actually enhanced by IgG antibody. For example, enteroviruses are susceptible to antibody-mediated opsonization, while replication of arboviruses may be enhanced by antibody.

5. Enzyme inhibition—Influenza virus has a sur-

face protein, neuraminidase, which enzymatically cleaves the viral receptor (N-acetylneuraminic acid) from the host cell membrane. Neuraminidase has no known role in initiating viral infection of cells but does function by facilitating *release* of progeny virus. Antibody against neuraminidase has been shown to limit viral replication and spread but not to neutralize the virion. Presumably, similar nonneutralizing antigen-antibody interactions may be found for other viruses.

B. Cell-Mediated Defense Mechanisms: Delayed hypersensitivity to many viruses can be identified in the immune host using skin test reactivity (eg, mumps) or in vitro measures such as a blastogenic response of lymphocytes to viral antigens. Some cell-mediated reactions which appear to be important in host resistance to or defense against viral infection are as follows:

1. Cellular cytotoxicity–Virus-infected cells may be lysed by sensitized lymphocytes or in some cases by activated macrophages or even polymorphonuclear leukocytes. Virus-infected cells develop new antigens on their surfaces that are recognized as "nonself" by leukocytes, triggering lysis of the cell. Production of new cell surface antigens is particularly evident with enveloped viruses, such as the herpesviruses and myxoviruses, in which the viral envelope is derived by budding from an altered cell membrane. However, cellular cytotoxicity also has been shown with nonenveloped viruses (eg, reoviruses) that do not mature by budding. Where studied, these viruses have been shown to induce cell surface changes as well.

Cell-mediated cytolysis of virus-infected cells is mediated through 3 distinct mechanisms: (1) cytotoxic T cells, (2) natural cytotoxicity, and (3) antibody dependent cell-mediated cytotoxicity (Table 13–1). Cytotoxic T lymphocytes appear early in virus infection, recognize specific viral antigens, and require class I MHC compatibility between effector and target cells for cytolysis. The effector T cells are of the suppressor-cytotoxic subset, as defined by monoclonal antibodies. The protective effect of cytotoxic T cells has been proved in mice by adoptive transfer experiments.

Table 13–1. Distinguishing features of antiviral cytolytic reactions.

Descriptive Term for Cytolysis	Effector Cell(s)	Target Antigen Specificity?	Class I MHC Restriction?
Cytotoxic T lymphocytes	Subclass of T lymphocytes	Yes	Yes
Natural cytotoxicity	NK cells (Fc receptor-bearing T lymphocytes and macrophages)	No	No
Antibody-dependent cell-mediated cytotoxicity (ADCC)	K cells–T lymphocytes, macrophages, and perhaps other leukocytes with Fc receptors	Yes (through antibody)	No

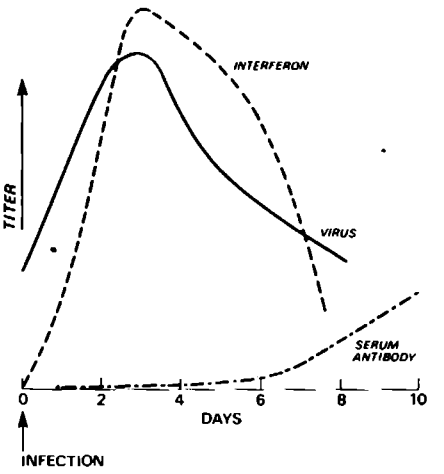

Figure 13–6. Appearance of interferon and serum antibody in relation to recovery from influenza virus infection of the lungs of mice. (From Isaacs A: *New Scientist* 1961; 11:81.)

The few studies performed to evaluate the cytotoxic T cell response in human viral infections suggest that a diminished response is associated with more severe or prolonged infection.

Natural cytotoxicity is mediated by many types of cells (so-called NK cells) bearing Fc receptors, including some T cells and macrophages (see Chapter 7). The cytotoxicity is "natural" in that it is present toward antigens to which the animal has not previously been exposed. Susceptible targets include virus-infected autologous or heterologous cells and various transformed and tumor-derived cell lines. There is no requirement for HLA matching of effector and target cells. Antiviral NK activity is markedly enhanced by interferon, and the increased NK activity that follows most viral infections closely parallels the interferon response (Fig 13–6). Animal experiments support a role for NK cells in resistance to virus infection, but data for humans are lacking.

Antibody-dependent cellular cytotoxicity is mediated by cells similar to NK cells but requires antibody coating of the target cells. The specificity of the reaction is determined solely by the antibody used, with lysis occurring when the effector cells bind the Fc fragment of IgG that is bound to the target cell. There is no requirement for cellular recognition of the antigens or MHC matching between effector and target cells. Antibody-dependent cellular cytotoxicity has been demonstrated in vitro with human and animal cells, and passive transfer experiments in animals show an important role for this immune mechanism in the limitation of virus infection. However, data supporting the role of this phenomenon in resistance of humans to virus infections are lacking.

2. Lymphokine production–Sensitized lymphocytes release lymphokines in response to viral antigens. The best characterized of these from a virologic

standpoint is **interferon** (see below). Lymphokines also attract effector cells (macrophages and polymorphonuclear leukocytes) that may nonspecifically destroy viruses or virus-infected cells; some lymphokines are directly toxic to virus-infected cells.

3. Interferon—Interferon is a family of proteins produced by somatic cells in response to a variety of stimuli, including virus infection. These proteins were identified originally by their potent antiviral activity, but more recently they have been shown to affect a multiplicity of cellular functions, including the immune response. Additionally, interferons inhibit the growth of some other intracellular parasites (eg, *Chlamydia*) and certain tumor cells.

Recently, 3 clearly distinct types of interferon have been recognized and the nomenclature codified by an expert committee (Table 13–2). Alpha and beta interferons are primarily antiviral, while the function of gamma interferon is primarily immunoregulatory. However, these immunoregulatory activities (eg, augmentation of leukocyte cytotoxicity) may affect the outcome of virus infections as well.

Interferon production may be stimulated by infection by viruses, protozoa (eg, *Toxoplasma*), rickettsiae, chlamydiae, and certain bacteria. A variety of bacterial products, such as endotoxins and nucleic acids, also are interferon inducers. In addition, synthetic nucleic acids (especially double-stranded RNA) and some chemicals (eg, tilorone) may stimulate interferon production. Sensitized lymphocytes will make immune interferon in response to specific antigens, including viral antigens.

Interferon is produced by and released from cells early in the course of virus infection (in some cases, at the time of adsorption); thus, it is available much earlier than antibody (Fig 13–6). The antiviral action of interferon is mediated indirectly, through effects on host cells. Exposure of cells to interferon triggers intracellular synthesis of antiviral proteins that probably act by selectively inhibiting the synthesis of viral proteins (Fig 13–7). These antiviral proteins (or other effector molecules) also alter a variety of other cell functions. The antiviral activity may be transferred to neighboring cells, without the continued presence of interferon, via an unknown mechanism. The antiviral state persists after interferon exposure and is lost slowly, probably through cell death or division. The antiviral action of interferon is pathogen-nonspecific but relatively host-specific. Although interferon production is stimulated by many viruses and inhibits the growth of many viruses, the protection it gives is limited to the cells of the producing species. For example, human interferon will protect human and some primate cells but will not protect mouse or chicken cells.

The evidence that interferon is an important host defense against virus infection is summarized in Table 13–3. Perhaps the most persuasive evidence comes from animal models of virus infection in which the in-

Table 13–2. Characteristics of the recognized types of interferon.*

Interferon Type	Experimentally Demonstrated Property		
	Producer Cell	Inducing Stimulus	Major Activity
Leukocyte (alpha)	Null lymphocytes	Viruses, foreign cells	Antiviral, activate killer cells
Fibroepithelial (beta)	Fibroblasts, epithelial cells, macrophages	Viruses, nucleic acids	Antiviral
Immune (gamma)	T lymphocytes	Mitogens (including antigens)	Immunoregulatory

*Adapted, with permission, from Baron S: The interferon system. *Am Soc Microbiol News* 1979;45:358.

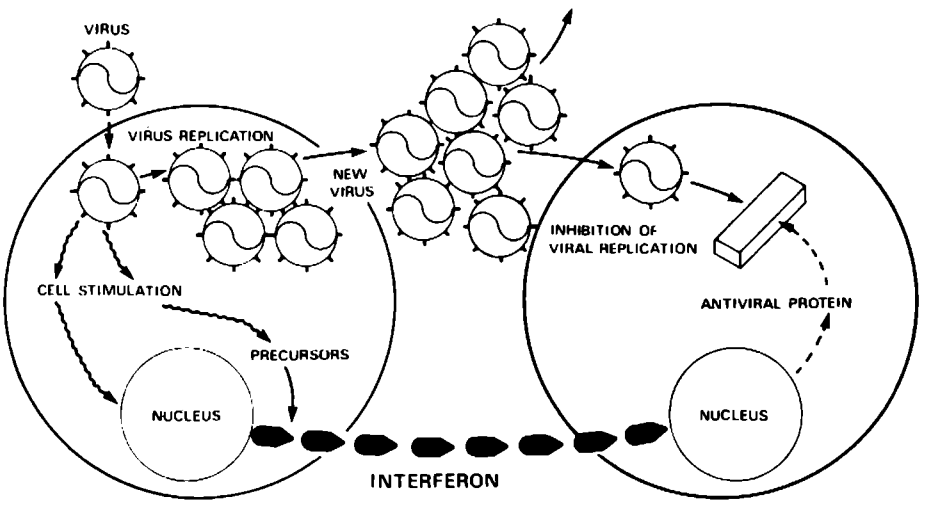

Figure 13–7. Schematic representation of interferon activity.

Table 13–3. Evidence for role of interferon in host defenses against virus infection.*

Type of Evidence	Example
Time correlation	Interferon produced just before and during arrest of virus replication, before specific response.
Place correlation	Maximal interferon production near site of maximal virus replication.
Quantitative correlations	Concentrations of interferon produced in vivo are comparable to amounts required in vitro for antiviral activity.
Transfer	Exogenous or chemically induced interferon controls virus infections in humans and experimental animals.
Ablation	Deletion of interferon (eg, by specific antibody) in cell culture and experimental animals enhances virus replication.

*Adapted, with permission, from Baron S: The interferon system. *Am Soc Microbiol News* 1979;45:358.

terferon response was specifically ablated using anti-interferon antibody. The relative contribution of interferon to host control of virus infection varies with the system studied, but it appears to play a significant role in most.

Recently, highly purified interferon has become available in large quantities. Initially, it was derived from human peripheral blood leukocytes, but more recently it has been produced using recombinant DNA technology. Clinical trials with natural and recombinant leukocyte interferon have demonstrated beneficial results in human infections due to herpesviruses (herpes simplex, varicella-zoster, and cytomegalovirus), rhinoviruses, hepatitis B virus, and papillomaviruses (warts). The toxicity of the natural and recombinant products is similar and includes fever, malaise, myalgia, enervation, leukopenia, and thrombocytopenia. The side effects are sufficiently common and severe that leukocyte interferon is unlikely to be used for mild infections. Clinical trials with recombinant immune (gamma) interferon are just beginning.

4. Host restriction—In the case of several viruses, virulence has been associated with ability to replicate in macrophages. For example, with mouse hepatitis virus, virulent strains can replicate in macrophages whereas avirulent strains do not. Conversely, macrophages from strains of mice resistant to mouse hepatitis virus infection or illness generally are nonpermissive of viral replication, whereas macrophages from susceptible strains allow virus replication. Host restriction may be mediated in part by the immune response. For example, rabbitpox virus replicates in rabbit macrophages, but if the macrophages are activated (eg, by lymphocyte supernates), virus replication no longer occurs.

5. Phagocytosis—Phagocytosis of viruses by macrophages or, less commonly, by neutrophils (especially in the presence of antibody) may be responsible for resistance to infection and illness (eg, with enteroviruses).

C. Other Host Defenses: Many nonspecific factors have been shown to alter the outcome of virus infection in experimental systems. For example, body temperature (fever or hypothermia) and local hypoxia or acidosis might affect virus replication. Some of these factors may act by affecting host defense mechanisms discussed previously, as well as by directly altering virus replication. In many cases, however, the role these factors play in the intact animal is obscure.

Experimentally, some viral infections (especially herpesviruses) can be shown to be modulated almost entirely by cellular immunity, with antibody playing no demonstrable role. Despite this, the exact component of cellular immunity (cellular cytotoxicity, phagocytosis, lymphokine production) which is responsible for protection has not been defined in most cases.

Mechanisms of Virus-Induced Cell & Tissue Injury

Viruses may cause cell and tissue injury either directly or indirectly through interaction with various host factors, especially the immune system.

A. Direct Injury: Many viruses can damage cells directly, in the absence of host factors. Injury occurs in many instances because virus infection of the cell is associated with inhibition of metabolic processes (eg, protein synthesis) necessary for cell integrity. Examples of directly cytolytic viruses include herpes simplex and adenoviruses. Recently, investigators have documented direct virus-induced cell injury that is not cytolytic but results in more subtle defects in synthetic functions. For example, cultured chick melanocytes infected with Rous sarcoma virus appear grossly normal and remain viable, yet synthesis of melanin ceases.

B. Indirect Injury:

1. Humoral—Virus-infected cells may be lysed by antibody and complement or by complement alone, as described above. However, the evidence that this mechanism contributes to virus-induced disease in vivo is sparse. Virus infections may trigger production of autoantibodies that cause autoimmune disease. Relatively subacute infections by noncytolytic viruses may result in virus-induced immune complex disease. In these instances, nonneutralizing antiviral antibody combines with virus, and the resulting immune complexes are deposited in tissues. Tissue damage occurs through the mediation of complement and leukocytes attracted to the immune complexes. The best example of this phenomenon in humans is the vasculitis that occurs with hepatitis B infection; numerous examples are known in animal models (lactic dehydrogenase virus, lymphocytic choriomeningitis virus). Some virus infections (eg, Epstein-Barr virus infection of humans) are associated with polyclonal B cell proliferation and hyperglobulinemia. Some of these immunoglobulins may be directed against autoantigens, likewise causing immune complex disease. Nonneutralizing antibody may enhance replication of viruses, especially those which replicate in macrophages or other Fc receptor-bearing cells. The clinical relevance

of this phenomenon has been demonstrated most conclusively with dengue virus, where second infections (occurring in the presence of antibody) result in higher level viremia and greater illness than initial infections. The macrophage is a major site of dengue virus replication, and antibody enhances virus infection and replication within this cell.

2. Cell-mediated—Host cell injury may occur via the cellular effector mechanisms described above. An in vivo role for this immunopathologic mechanism is suggested by animal experiments showing prolongation of survival (but a higher number of deaths) in immunosuppressed mice as compared with normal mice following infection with certain viruses.

ANERGY & INFECTION

The mechanisms responsible for cell-mediated immunity are complex and potentially vulnerable to disruption by a variety of processes. Inability to express delayed hypersensitivity to tuberculin has been appreciated in sarcoidosis and Hodgkin's disease for over 50 years. We now know that anergy extends to a variety of antigens and, in the case of Hodgkin's disease, is associated with a predisposition to opportunistic intracellular infection. The mechanism by which these 2 diseases disturb cell-mediated immunity is not known, but both conditions are characterized by a granulomatous reaction that may extensively invade the paracortical areas of lymph nodes and the white pulp of the

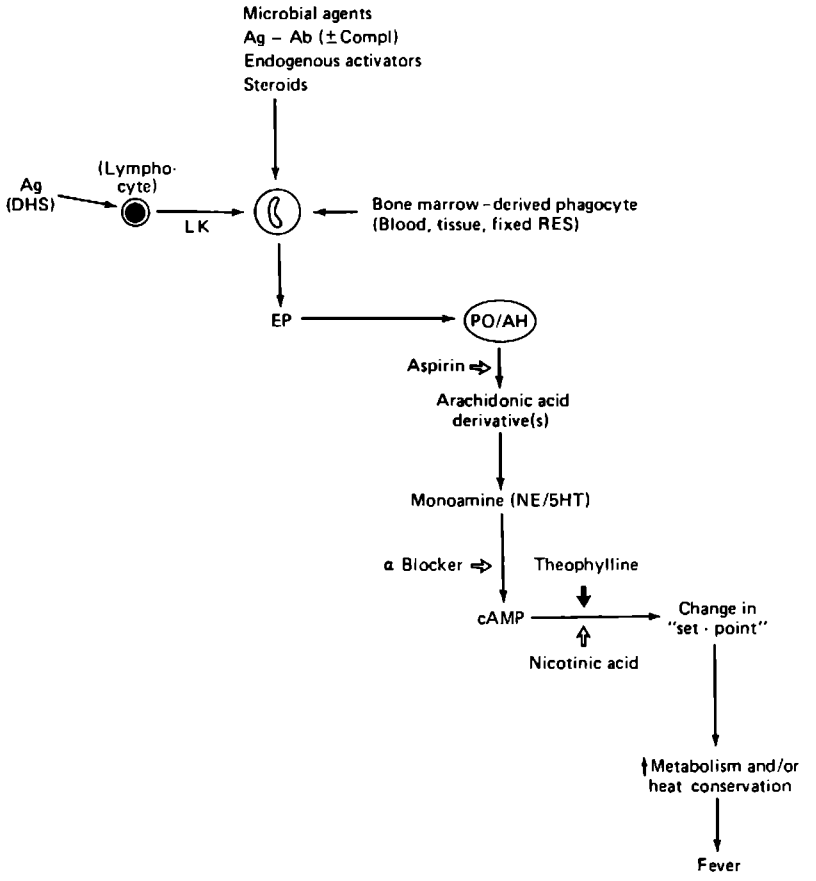

Figure 13–8. Postulated pathway for the pathogenesis of fever. Endogenous pyrogen circulates to the hypothalamus (PO/AH), where it induces the production of a metabolite of arachidonic acid. This substance, in turn, increases the synthesis of norepinephrine, an alpha-adrenergic agonist that increases the production of cAMP. This cyclonucleotide then directly causes alterations in the activity of temperature-sensitive neurons that bring about an increase in heat conservation or production (or both). Ag = antigen; Ag-Ab = antigen-antibody complexes; Compl = complement; cAMP = cyclic 3',5'-adenosine monophosphate; DHS = delayed hypersensitivity; EP = endogenous pyrogen; 5HT = serotonin; LK = lymphokine; NE = norepinephrine; PO/AH = preoptic area of anterior hypothalamus; RES = reticuloendothelial system; ⇨ inhibitors; → activators. (Reproduced, with permission, from Bernheim HA, Block LH, Atkins E: Fever: Pathogenesis, pathophysiology, and purpose. *Ann Intern Med* 1979;91:261.)

spleen. It is to these areas that T lymphocytes normally migrate and proliferate when antigenically stimulated.

Viral Diseases

Certain viruses can infect cells of the immune system and thereby impair their function. In 1911, von Pirquet noted that the tuberculin skin test reaction disappeared during the first days of rash with measles infection. In fact, tuberculin anergy may persist up to 2 months beyond the appearance of the rash. Both delayed hypersensitivity and cell-mediated immunity are suppressed by measles; quiescent cases of tuberculosis may be reactivated and existing cases worsened by infection with this virus. This effect may be due to damage to either lymphocytes or mononuclear phagocytes. Although other viral infections (influenza, varicella, type 1 poliovirus vaccine) can depress skin reactivity to tuberculin, at least partly on the basis of lymphocyte cytotoxicity, these infections do not appear to predispose to reactivation of latent infections due to intracellular pathogens.

Immunodepression by mumps virus appears to result from a nonspecific inhibition of sensitized lymphocytes or effector macrophages involved in the delayed hypersensitivity reaction. Thus, skin sensitivity to various antigens can be successfully transferred to normal recipients from previously sensitized guinea pigs with active mumps infection even though the infected guinea pigs are anergic at the time of cell transfer.

Recent studies suggest that development of fatal wild rabies infection may reflect the operation of a selective immunosuppressive mechanism whereby cytotoxic T cells specific for rabies virus-infected target cells fail to develop. Such cells do develop during nonfatal infection with attenuated strains of rabies virus. Wild rabies virus is not known to replicate in the cells of the immune system.

Since recirculation of T cells appears essential to facilitate the induction and expression of cell-mediated immunity, infection by viruses that can disrupt this traffic may damage the immune reactivity of the host. For example, thoracic duct lymphocytes (which are predominantly T cells) have specific receptors for myxo- and paramyxoviruses. Newcastle disease virus (a paramyxovirus) can attach to sialyl residues on the lymphocyte surface and then elute, destroying the receptors and releasing sialic acid. This results in a temporary but severe disruption of lymphocyte recirculation from blood to lymph, because cells normally homing to the spleen and lymph nodes are diverted and trapped by the liver. Another mechanism of immunosuppression occurs in infections with lactic dehydrogenase (LDH) virus, which produces cytopathologic changes in thymus-dependent areas of the spleen, the paracortical areas of lymph nodes, and the cortex of the thymus. In addition to depressing cell-mediated immunity, the presence of chronic viremia results in increased numbers of germinal centers (the sites of B cell proliferation) and enhanced antibody response to certain antigens.

Granulomatous Infections

Lepromatous leprosy, miliary tuberculosis, disseminated coccidioidomycosis, and other disseminated chronic intracellular infectious diseases are associated with both specific and nonspecific suppression of cell-mediated immunity. Specific immunosuppression is generally marked by failure to respond to antigens derived from the infecting microorganism (eg, lepromin in patients with leprosy; coccidioidin or spherulin in coccidioidomycosis; tuberculin in patients with miliary tuberculosis). Nonspecific immunosuppression is manifested by prolongation of skin homografts, depression of mixed lymphocyte responses, anergy to unrelated skin tests, and decreased sensitization to hapten-sensitizing agents such as DNCB. Whereas antimicrobial therapy can generally reverse the nonspecific immunosuppression, specific anergy to the infecting microorganism is often not reversible (particularly in leprosy). Thus, the nature of the 2 immunosuppressive phenomena may not be identical, and unrestricted proliferation of microorganisms due to the specific immune defect may lead secondarily to the nonspecific defects.

The mechanism of nonspecific immunosuppression is not fully understood. However, there is evidence that the presence of multiple granulomas in the white pulp of the spleen and in paracortical areas of lymph nodes may divert lymphocyte traffic, as in Hodgkin's disease. Alternatively, these diseases may be associated with serum or plasma factors that suppress lymphocyte function in vitro. Such factors tend to disappear when the antigenic load is reduced by chemotherapy. Finally, the defect may result from a suppression of cell-mediated immune responses by monocytes or by non-IgG-bearing, nonphagocytic, sheep red cell rosette-forming cells that appear to be suppressor T cells. Such suppressor T cells might be generated in response to the specific challenge organism.

The mechanism of specific immunosuppression is even less well understood. However, some data suggest that a relationship to Ir genes may exist. For example, variation in susceptibility to acute infection with lymphocytic choriomeningitis virus in adult mice is partially controlled by dominant genes closely linked with the H-2 locus. Recent studies suggest that there is a relationship between coccidioidal dissemination and type 9 histocompatibility specificity. Whether this association is independent of HLA prevalence within racial groups at increased risk (blacks, Filipinos) is unclear at this time.

FEVER

The occurrence of fever in infectious diseases is so universal that its presence generally stimulates a search for an invading pathogen. In only a few infections (eg, gonorrhea, syphilis, lepromatous leprosy) is fever absent. Despite its nearly universal occurrence and its historical association with infection, the true significance of fever remains uncertain. Nevertheless,

there is at least some evidence that it plays a role in host defense. For example, human lymphocytes incubated in vitro at 39 °C show a higher uptake of thymidine than lymphocytes incubated at 37 °C. Also, human leukocytes demonstrate maximal phagocytic activity between 38 and 40 °C. Finally, during fever there is a decrease in circulating levels of iron. This observation, coupled with the fact that the production of bacterial siderophores (iron-binding proteins) is suppressed by elevated temperature, may indicate that fever hinders the ability of microorganisms to obtain and sequester needed supplies of iron. Although fever has never been shown to have frank survival implications for mammals, it has been shown clearly that cold-blooded animals such as the desert iguana will perish from certain infections unless allowed access to elevated environmental temperatures. (Only by moving to a warm environment can such animals raise their body temperature.) Infected febrile lizards become hypoferremic; when injected with exogenous iron, their mortality rate increases significantly despite their being kept at a warm ambient temperature.

Current concepts of fever production indicate that the common stimulus to body temperature elevation is the response of the preoptic area of the anterior hypothalamus (PO/AH) to a circulating substance known as leukocytic pyrogen or endogenous pyrogen (EP). Endogenous pyrogen is a protein that is freshly synthesized or cleaved off from an active precursor in bone marrow-derived phagocytes in response to microbial agents, antigen-antibody complexes, endotoxin (lipid A component), or the pyrogenic steroid etiocholanolone. Human monocytes and macrophages are capable of generating this pyrogen, whereas lymphocytes are not. However, it is known that lymphocytes may produce soluble factors that enhance pyrogen production by macrophages. There is evidence that the pyrogen's action on the anterior hypothalamus is mediated through the activities of monoamines, sodium, calcium, prostaglandins, and cAMP. Fig 13–8 illustrates one scheme whereby a febrile response is produced.

Endogenous pyrogen has recently been highly purified, and a radioimmunoassay has been developed for its detection. Studies have also shown that endogenous pyrogen is the same molecule as interleukin-1 (IL-1). This implies a far broader immunoregulatory role for endogenous pyrogen than simply regulating the febrile response.

In vitro, endogenous pyrogen is capable of stimulating the respiratory burst in PMNs and stimulating the reduction of NBT dye to formazan. These observations help in understanding the phenomenon of increased spontaneous NBT dye reduction in the leukocytes from patients with a variety of disease processes having fever as their only common feature. In addition, the protein has been shown to cause selective release of specific granule contents (lysozyme and lactoferrin) from human neutrophils in vitro. It is possible that the purpose of lactoferrin release is restriction of iron availability to microorganisms. The function of lysozyme release is less clear, but it is known that neutrophils from patients with acute bacterial infections may contain 50% less lysozyme than control neutrophils.

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14 Tumor Immunology

Philip D. Greenberg, MD

Tumor immunology is the study of the antigenic properties of transformed cells, the host immune responses to these tumor cells, the immunologic consequences to the host of the growth of malignant cells, and the means by which the immune system can be modulated to recognize tumor cells and promote tumor eradication. One potentially important function of an organism's immune system is to provide protection from the outgrowth of malignant cells. This represents a formidable task, since tumor cells have many similarities with normal cells despite exhibiting abnormal propensities to proliferate, spread throughout the host, and interfere with normal organ functions. Thus, tumor cells present special problems to the host immune system beyond those presented by other self-replicating antigens such as bacteria, which can be more easily distinguished as foreign. Elucidating the processes that render a cancerous cell different from normal cells should aid in understanding how these transformed cells might be amenable to destruction or regulation by the immune system.

Normal cells have a variable capacity to proliferate and to express differentiated function. These cell activities are tightly coordinated within an organ or tissue so that the rate of cell loss (due to the natural death of mature differentiated cells) is equal to the rate of appearance of new cells from the less mature proliferating cell pool. If the stimulus for cell proliferation exceeds the requirement for cell replacement, as occurs in some pathologic conditions, the organ hypertrophies as a result of polyclonal expansion of cell number. However, if the stimulus for cell growth is removed, the rate of cell proliferation will decrease and organ hypertrophy will be reversed. In contrast to this nonmalignant regulated polyclonal cell growth, an individual cell may undergo a transforming event and acquire the potential to produce daughter cells that can proliferate independently of external growth signals. The autonomous growth of such transformed cells of monoclonal origin represents the basis of malignant disease. Many of the properties of tumor cells are summarized in Table 14–1. The protean effects of cancer on the host are in large part a reflection of the unrestrained growth of tumor cells that can locally invade and disrupt normal tissue as well as metastasize and grow in distant organs.

DEVELOPMENT OF TUMORS

The transformation of a normal cell into a malignant one can result from a variety of events, the specific nature of which may help to determine if the immune system can control the outgrowth of the tumor cells. These transforming events may occur spontaneously by random mutations or gene rearrangements, or they may be induced by a chemical, physical, or viral carcinogen.

Chemical Carcinogens

Tumors induced by chemical carcinogens were initially described in the 18th century when chimney sweeps, who worked in direct contact with soot and tar, were observed to have a high incidence of carcinoma of the scrotum. Polycyclic aromatic hydrocarbons in soot and tar have since been found to be potent carcinogens, and retention of tar in the wrinkles of the scrotum was apparently responsible for the neoplastic transformation. In fact, painting tar on epithelial cells has subsequently been used for induction of tumors in the laboratory. Several distinct polycyclic hydrocarbon carcinogens have been identified, including benzpyrene, methylcholanthrene, and aflatoxin. Another major class of carcinogens, the aromatic amines, was identified following the observation of a high frequency of bladder cancer among factory workers using aniline dyes. Many other environmental carcinogens have also been identified, including nitrates used as food preservatives, asbestos used as an insulating material, and vinyl chloride used in plastics.

Most environmental carcinogens do not appear to directly affect gene function. However, during the in vivo catabolism of these compounds, intermediate

Table 14–1. Common properties of tumor cells.

Failure to respond to the regulatory signals responsible for normal growth and tissue repair.
Autonomous growth without an absolute requirement for exogenous growth signals.
Invasive growth through normal tissue boundaries.
Metastatic growth in distant organs following entry into blood and lymph channels.
Monoclonal origin, although genotypic and phenotypic heterogeneity may become detectable as tumor mass increases.
Differences in appearance and membrane antigenic display from nontransformed cells of the same tissue origin.

products can be formed that do have direct mutagenic activity. For example, detoxifying enzymes such as the aryl hydrocarbon hydroxylases convert the polycyclic aromatic hydrocarbons and aromatic amines to active intermediate compounds prior to esterification for excretion. Situations in which the active intermediates accumulate to carcinogenic levels include heavy exposure to the carcinogen or genetically determined high levels of aryl hydrocarbon hydroxylases and have been associated with an increased risk for development of cancer.

Physical Carcinogens

Evidence of the carcinogenic potential of ionizing radiation accrued rapidly following the realization that many of the early radiologists who developed radiodermatitis following acute exposures eventually developed skin cancer after a long latency period. Increased rates of bone cancers were also observed in workers who inadvertently ingested radium while applying luminous paint to watch dials. The most dramatic evidence of radiation-induced carcinogenesis was in survivors of the atomic bomb explosions in 1945 in Japan, who demonstrated an increased incidence of a wide range of tumors for more than 20 years afterward. The long latency period observed for the development of a neoplasm following exposure to ionizing radiation and the nonlinear relationship between dose of radiation and risk of tumorigenesis suggest that malignant transformation reflects more than a "one-hit" process. Radiation presumably directly injures cellular DNA, resulting in mutations, chromosomal breaks, and abnormal rearrangements, but the expression of malignancy in radiation-damaged cells requires the presence of additional genetic events.

An association between exposure to sunlight and development of skin cancer has been documented for more than a century. The carcinogenic potential of ultraviolet radiation is implied by the more frequent occurrence of skin cancer on sun-exposed parts; the inverse correlation between pigmentation of the skin, which filters out ultraviolet light, and the incidence of skin cancer; and the increased incidence of skin cancer in people occupationally exposed to sunlight or living in areas with more intense sunlight. People with the inherited disease xeroderma pigmentosum have a defective repair mechanism for ultraviolet light-induced damage to DNA and exhibit an increase in skin cancer. More direct evidence has been obtained by exposing mice to ultraviolet rays and observing the appearance of skin cancers. The mechanisms of carcinogenesis by ultraviolet irradiation may be numerous, including production of carcinogenic oxide metabolites, induction of pyrimidine dimers, and immunosuppression.

Viral Oncogenesis

Although the potential for viruses to induce tumors has been evident from studies in animals since the beginning of this century, the relevance of this finding to human disease was heavily debated until human oncogenic viruses were finally identified. Virus-induced tumors are of particular interest in tumor immunology, because of the great likelihood that cells transformed by the introduction of viral genes will express new virus-associated antigens that can be recognized by the immune system. Oncogenic viruses can be subdivided into either DNA or RNA types, based on the genetic information carried by the intact virus. Most cells infected by the potentially oncogenic DNA viruses (which include papovaviruses, herpesviruses, and adenoviruses) do not become transformed. Following infection of such permissive cells with these DNA viruses, the viral genome directs the cell's biosynthetic machinery to replicate viral DNA and assemble viral particles, culminating in cell lysis with release of infectious virions. By contrast, infection of nonpermissive cells can result in integration of the viral DNA into the host genome and expression of only some of the viral genes, so that lytic virus particles are not formed. Transformation may result either from direct triggering of host genes by the integrated viral DNA or from the host aberrantly splicing viral messages to produce new proteins that promote transformation. SV40 is the best studied DNA oncogenic virus. Cells transformed by SV40 contain integrated copies of the viral genome, manufacture proteins coded by viral genes, and express virus-associated tumor proteins that are immunogenic to hosts bearing SV40 induced tumors. Although no DNA viruses with direct oncogenic potential have been isolated from human tumors, several cancers exhibit marked statistical linkage with prior or concurrent evidence of particular viral infections, including Epstein-Barr virus (EBV) infection with both Burkitt's lymphoma and nasopharyngeal carcinoma, herpes simplex virus (HSV) type 2 infection with cervical carcinoma, and hepatitis B virus (HBV) infection with primary liver cancers.

Oncogenic RNA viruses contain genes for a polymerase called reverse transcriptase that permits use of viral RNA as a template for transcription of a single-stranded DNA copy and promotes subsequent conversion of this DNA copy to a double-stranded form which can be inserted into the host genome. This process represents a reversal of the normal DNA to RNA transcription of genetic information, and these viruses are therefore often referred to as **retroviruses.** RNA tumor viruses were first discovered by Ellerman and Bang in 1908 and Rous in 1911 from tumor filtrates of a chicken leukemia and a chicken sarcoma, respectively. Retroviruses have now been shown to be responsible for a large number of naturally occurring malignancies in many species. A class of human retroviruses, the human T leukemia viruses (HTLV), have now been identified as being responsible for certain T cell leukemias and lymphomas. Many retroviruses, such as feline leukemia virus (FeLV) and HTLV, readily spread horizontally from infected to normal hosts, and resistance to tumorigenesis appears to be in part dependent upon the generation of an immune response to virus-associated antigens.

Integration into the host genome of the complementary DNA to the RNA of retroviruses results in the

production of new infectious viruses that bud from the cell membrane (so-called C-type particles), expression of structural and other virus-associated antigens on the cell membrane, and potentially transformation. Retroviruses can be separated into rapidly transforming and slowly transforming types. Rapidly transforming viruses contain oncogenic genes that can directly transform cells, whereas slowly transforming viruses lack such oncogenes and must aberrantly activate host genes that in turn induce transformation. Regardless of the mechanism by which retroviruses induce tumors, these tumors are likely to express new surface antigens that can be recognized by the immune system.

Cellular Oncogenes

Analogs to many of the viral oncogenes have been identified in the normal cellular genome, and in vitro studies have demonstrated that under appropriate conditions activation of these cellular oncogenes can transform normal cells. The functions of cellular oncogenes in normal cell growth and development remain to be elucidated, but it is presumed that changes which result in maintaining these genes in a transcriptionally active state will result in transformation. This may occur following mutation, translocation to a site adjacent to an active gene, or insertion of a promoter such as a slowly tranforming retrovirus. The known oncogenes code for a wide variety of products including protein kinases, nuclear and membrane proteins, growth factors, and membrane receptors. It has already been demonstrated that expression of at least some of these oncogene products will render malignant cells sufficiently disparate from normal cells for distinction immunologically.

ANTIGENS ON TUMOR CELLS

The field of tumor immunology is based in large part on the assumption that tumors express antigens which permit immunologic separation of malignant from normal cells. The initial attempts to identify tumor antigens mistakenly identified histocompatibility antigens and led to a great deal of skepticism about the existence of tumor antigens. Studies with experimental animal tumors as well as spontaneous human tumors have now demonstrated that many tumors do express antigens which can induce cellular or humoral responses in the host. The relevant tumor antigens fall into 2 major categories. Unique tumor-specific antigens are found only on tumor cells and therefore represent ideal targets for immunologic attack. By contrast, tumor-associated antigens are found on tumor cells and on some normal cells. However, qualitative and quantitative differences in antigen expression permit use of these antigens to distinguish tumor cells from normal cells. Host responses to tumor antigens are obviously much more apt to occur with unique tumor antigens.

The first studies to demonstrate the presence of unique tumor antigens utilized transplantable murine tumors induced by the chemical carcinogen methylcholanthrene (MCA). A sarcoma was induced with MCA in a primary host, completely resected before it had disseminated, and transplanted into a syngeneic secondary host (Fig 14–1). After outgrowth of the transplanted tumor in the secondary host, the tumor was again resected and transplanted both into a normal syngeneic mouse and back into the original host, which had been cured by surgical removal of the tumor. Progressive tumor growth was observed in the normal syngeneic mouse, but the original host from which the tumor was derived rejected the tumor inoculum. Subsequent studies demonstrated the immunologic specificity of this response for the tumor to which the animal had been exposed. Thus, tumors induced by MCA were shown to express unique tumor antigens immunogenic to the host. These studies also identified a major problem facing tumor immunologists—

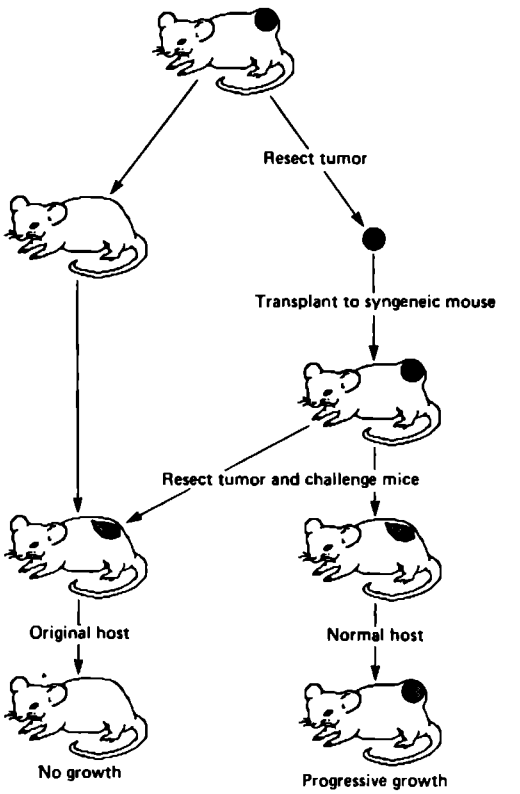

Figure 14–1. Tumors induced by a chemical carcinogen generate a host immune response. A methylcholanthrene-induced tumor was resected after it became palpable and transplanted into a syngeneic mouse. After the transplanted tumor became palpable, it was resected and transplanted into both a normal syngeneic mouse and the original host cured by surgical resection. Progressive tumor growth became evident in the normal recipient, but the original mouse rejected the tumor inoculum. This tumor rejection was immunologically specific and mediated by T cells.

namely, the failure of the host to successfully resist progressive tumor growth following initial challenge with an immunogenic tumor, despite the fact that the host generates an immune response that can be detected if the tumor is resected and the host is rechallenged with the same tumor. The causes of this ineffective host response to a primary tumor challenge and methods capable of enhancing it are discussed later in this chapter.

A wide variety of tumor antigens have now been described on both spontaneous and experimentally induced tumors. Although expression of these tumor-associated antigens must reflect a transformation-related heritable change in the cells' genetic material, there are many distinct molecular mechanisms, as outlined in Table 14–2, that may result in the production of a tumor antigen. The most straightforward mechanism reflects a transforming event resulting in the production of a new protein, such as would occur following a retrovirus infection, with the introduction of new genetic material and subsequent expression of viral proteins. Altered expression of normal molecules could result from mutations that change protein structure. This has been described for both major and minor histocompatibility antigens on tumors induced by chemical carcinogens. Some tumor antigens may result from the uncovering of normally nonexposed determinants, such as is observed with some of the complex branching glycolipid antigens in which deletion of a branch may expose a new antigenic determinant. Tumor antigens may also result from the aberrant expression of fetal or differentiation antigens, such as is observed by the expression on human gastric carcinoma cells of ABO blood group antigens disparate from the host ABO blood type.

Unique Tumor Antigens

Unique tumor antigens can be detected only on tumor cells and not on other cells of the host. The best-studied unique tumor antigens are the new antigens expressed on tumors induced in inbred mice by oncogenic viruses and chemical carcinogens. Since the tumor antigens expressed on retrovirus-induced tumors are viral antigens rather than cellular antigens, tumors induced by the same virus are cross-reactive. Thus, immunization with one retrovirus-induced tumor will protect against challenge with the same tumor or a second tumor induced by the same virus (Fig 14–2). In fact, there are families of retroviruses, such as the murine Friend, Moloney, and Rauscher viruses, that induce immunologically cross-reactive transformed cells. Immunization of animals with tumors induced by any of these viruses or with the relevant viral antigens will provide protection from challenge with a tumor induced by any one of the viruses.

Table 14–2. Molecular mechanisms responsible for new tumor antigens.

Biosynthesis of a new molecule.
Alteration of the structure of a normal molecule.
Uncovering of normally protected molecules.
Incorrect assembly of multimeric antigens.
Aberrant expression of fetal or differentiation antigens.

Tumors induced by chemical carcinogens express unique and individually distinct tumor antigens. Unlike what was observed with virus-induced tumors, mice immunized to one MCA-induced tumor will resist rechallenge with that tumor but remain susceptible to challenge with a second tumor induced by MCA (Fig 14–2). In fact, multiple tumors induced with MCA in the same mouse will be immunologically distinct and will not elicit cross-protection if used to immunize and challenge syngeneic mice. The precise nature of the tumor antigens induced by MCA or other chemical or physical carcinogens has not been identified, but it is evident that there are an enormous number of potentially distinct tumor antigens. Since chemical carcinogens contribute significantly to the development of many human tumors, the presence of so many individually distinct tumor antigens is a mixed blessing. On the one hand, the expression of a distinct determinant on each tumor makes it unlikely that antigens can be identified which will be useful for immunizing the population to provide protection from tumor development. On the other hand, the presence of such antigens suggests that it may be possible to modulate host immunity to promote tumor eradication.

Analysis for the presence of unique tumor antigens has been more difficult with tumors developing in outbred species because of the inability to perform tumor transplantation studies. Molecular techniques have now permitted the isolation of retroviruses such as the HTLVs in humans and FeLV in cats, the identification of viral proteins, and the demonstration that these unique virus-associated antigens are expressed on retrovirus-induced tumors. "Spontaneous" tumors, many of which may have actually been induced by exposure to environmental carcinogens, have no predictable antigenic markers and therefore have been harder to study. However, recent technologic advances have made it possible to expand low-frequency antigen-reactive T cells and antibody-forming B cells from tumor-bearing hosts. Studies using these techniques have permitted the identification of apparent tumor-specific T cells and B cells in tumor-draining lymph nodes of patients with melanoma and breast cancer, as well as from lymph nodes following immunization with human colon carcinoma cells in association with an immunoadjuvant. Similar in vitro methods have been used to detect tumor-specific responses in a wide variety of apparently spontaneous human tumors, including leukemias, lymphomas, and lung cancer. Despite the presence of apparent tumor-specific lymphocytes, it has not yet been possible to isolate and characterize an unequivocal unique tumor antigen from these human tumors.

Tumor-Associated Antigens

Although it may not be possible to detect unique tumor antigens on all tumors, many tumors have an anti-

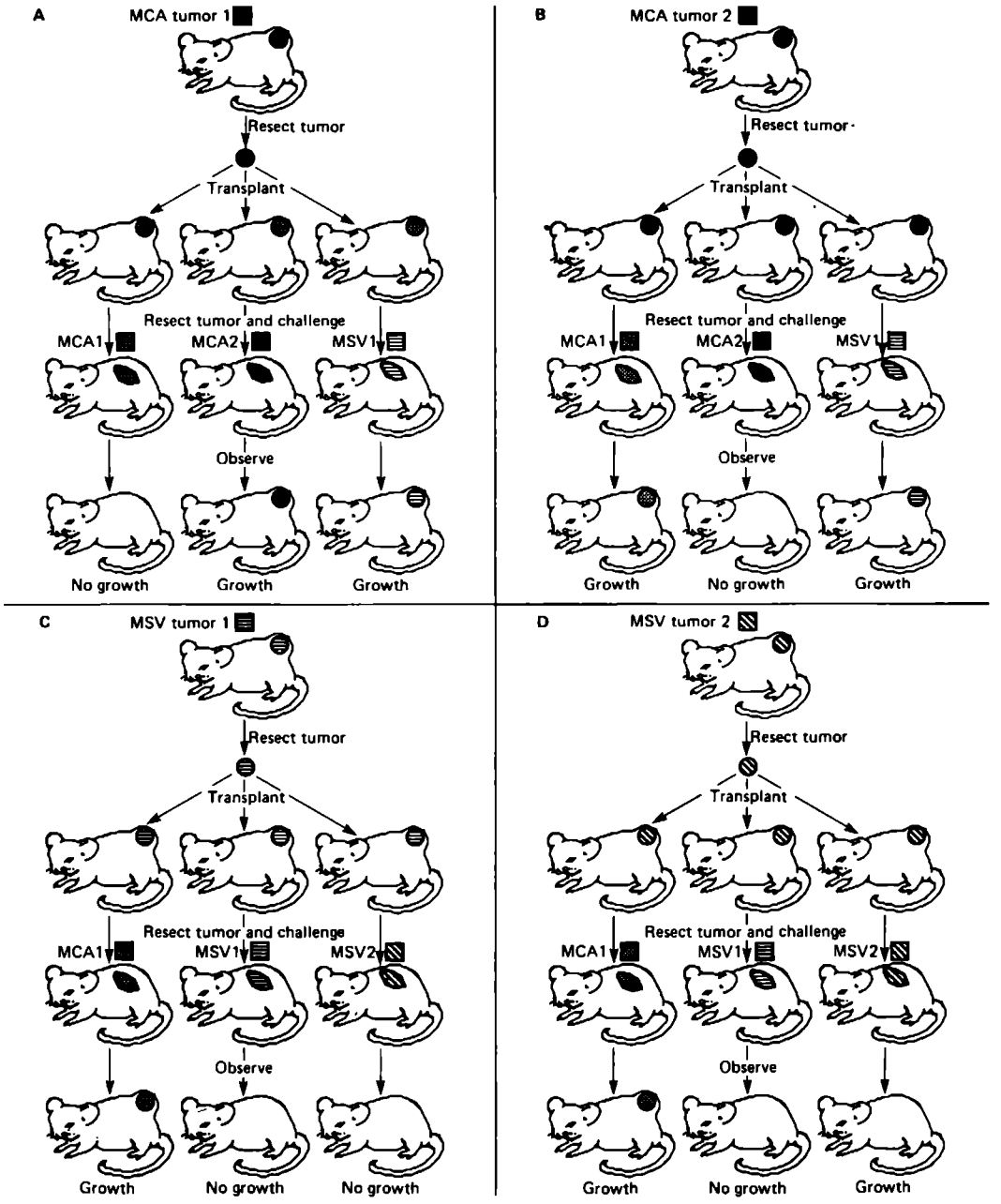

Figure 14–2. Tumors induced by the same chemical carcinogen express distinct tumor antigens, but tumors induced by the same retrovirus express cross-reactive tumor antigens. Tumors were induced in syngeneic mice with methylcholanthrene (MCA1 and MCA2) and with Moloney sarcoma virus (MSV1 and MSV2). The tumors were resected after they became palpable and were used for tumor transplantation studies. Mice immunized to MCA1 by resection of a palpable tumor *(A)* were found to reject a challenge with MCA1 but did not retard the growth of MCA2 or MSV1. Similarly, mice immunized to MCA2 by resection of a palpable tumor *(B)* rejected a challenge with MCA2 but did not retard growth of MCA1 or MSV1. Thus, the tumor antigens expressed on each MCA chemical carcinogen-induced tumor were distinct from each other and antigens on tumors induced by MSV retrovirus. Mice immunized with MSV1 by resection of a palpable tumor *(C)* were found to reject a challenge with either MSV1 or MSV2 but did not retard the growth of MCA1. Similarly, mice immunized to MSV2 by resection of a palpable tumor *(D)* rejected challenges with either MSV1 or MSV2 but did not retard the growth of MCA1. Thus, the tumor antigens expressed on each MSV retrovirus-induced tumor were cross-reactive with each other but distinct from tumors induced by the MCA chemical carcinogen.

genic display that permits distinction from normal cells. These tumor-associated antigens may be expressed on some normal cells at particular stages of differentiation. However, the tumor may differ either in the quantitative expression of the antigen or in the expression of the antigen with a particular lineage or differentiation markers. The identification of relevant tumor-associated antigens has progressed very rapidly with the advent of the technology for generating and screening monoclonal antibodies. These monoclonal reagents have permitted isolation and biochemical characterization of the antigens and have been invaluable diagnostically both for the distinction of transformed from nontransformed cells and for the definition of the cell lineage from which the transformed cell arose.

The best-characterized tumor-associated antigens are the oncofetal antigens. These antigens are expressed normally during certain stages of embryogenesis but are absent or very difficult to detect in normal adult tissue. The prototype antigen is **carcinoembryonic antigen (CEA),** which is a glycoprotein found on fetal gut and human colon cancer cells. CEA is not present on normal adult colon cells. Since CEA is shed from colon carcinoma cells and found in the serum, it was originally thought that the presence of this antigen in the serum could be used to screen patients for colon cancer. However, inflammatory lesions involving cells of endodermal origin (such as occur with colitis or pancreatitis) and other tumors (such as pancreatic and breast cancer) also are accompanied by elevated serum levels of CEA. Although measurement of serum CEA generally is not useful for diagnosing cancer, monitoring the fall and rise of CEA levels in colon cancer patients undergoing therapy has proved useful in predicting response to treatment. Several other oncofetal antigens have been useful for monitoring human tumors. In particular, **alpha-fetoprotein,** which is an alpha-globulin normally secreted by fetal liver and yolk sac cells, is found in the serum of patients with liver and germinal tumors and can be used as a marker of disease status.

Differentiation and lineage-specific antigens, which are present on normal adult cells, may be aberrantly expressed on some tumor cells. For example, a T cell antigen is commonly expressed on the malignant human B cells found in chronic lymphocytic leukemia, and a red cell blood group antigen is frequently found on human stomach cancer cells. Similarly, antigens normally expressed on thymocytes are frequently found on murine leukemias. These inappropriately expressed antigens are very useful for identifying transformed cells, and their unexpected presence on tumor cells may ultimately aid in deciphering the regulation and function of such antigens on normal differentiated cells.

Many other tumor-associated antigens, which have unknown function but very limited tissue distribution, have now been identified with monoclonal antibodies. These glycolipid and glycoprotein antigens, such as those identified on melanoma and acute lymphocytic leukemia cells, have already been shown to have great potential for the diagnosis and treatment of tumors.

IMMUNOLOGIC EFFECTOR MECHANISMS POTENTIALLY OPERATIVE AGAINST TUMOR CELLS

Virtually all components of the immune system have been shown to have the potential to contribute to the eradication of tumor cells. Immunologically specific effector responses are probably most important with highly immunogenic tumors, and nonspecific effector responses are presumably of greater significance with less immunogenic tumors.

T Cells

The T cell response is unquestionably the most important host response for the control of growth of antigenic tumor cells. T cell immunity to tumors reflects the function of the two T cell populations—class II-restricted T cells (Th), which mediate their effect by the secretion of lymphokines to activate other cells, and class I-restricted T cells (Tc), which mediate their effect largely by direct lysis of tumor cells.

The precise contribution of each T cell population to the antitumor response is variable. Since most tumor cells express class I but not class II MHC antigens, the Th subset cannot directly recognize these tumor cells. Therefore, these Th are dependent upon antigen-presenting cells, such as macrophages, to present the relevant tumor antigens in the context of class II antigens for activation. Once activated, these T cells secrete lymphokines (eg, IL-2), which in turn activate Tc, macrophages, NK cells, and B cells. Th can produce other lymphokines such as lymphotoxin or tumor necrosis factor, which may directly lyse tumor cells. The Tc subset is capable of directly recognizing and killing tumor targets by disruption of the target membrane and nucleus. However, effective Tc responses are generally dependent upon class II-restricted Th responses to provide the necessary helper factors (eg, IL-2) to activate and promote the proliferation of Tc.

Experimental models have been designed to evaluate the independent role of each T cell subset in the antitumor response. These models have generally utilized adoptive transfer of purified T cell subsets or T cell clones into tumor-bearing T-deficient hosts. In these studies, Th have been shown to promote eradication of tumors in the absence of any participation by Tc. These results have emphasized the importance of the recruitment and participation of non-T cell effector mechanisms in antitumor responses. Transferred Tc have generally had more limited antitumor activity than Th. However, if IL-2 was infused along with Tc or if selected clones of Tc that secrete IL-2 were infused, the transferred Tc were capable of exerting a potent antitumor effect. Since IL-2 can also induce Tc to secrete lymphokines such as IFN γ that activate other cells such as macrophages to become tumoricidal, it is possible that even the observed antitumor ac-

tivity of directly cytolytic Tc reflects contributions by non-T cell effector mechanisms.

B Cells & Antibody-Dependent Killing

A potential role for host antibody responses in tumor immunity has been suggested by the frequent detection of antibody in the serum of hosts bearing transplanted experimental tumors. Moreover, recent studies, in which hybridomas or cell lines are formed from B cells derived from lymph nodes draining human tumors, have suggested that spontaneous tumors may also elicit antibody responses to tumor-associated antigens. However, the importance of antibody responses in the control of tumor growth is unclear. For example, with tumors induced by retroviruses, immunization with virus can induce an antibody response that protects against the development of tumors induced by the virus, but the antibody response contributes little to the control of tumors that have already been induced. However, antibodies that have minimal activity against the primary tumor can bind to circulating tumor cells and may interfere with the establishment of distant metastases. Thus, antibody responses by the host to a growing tumor may help limit spread of the tumor but do not provide an effective response against the primary tumor without a concurrent contribution by T cells.

There are 2 major mechanisms by which antibodies may mediate tumor cell lysis. Complement-fixing antibodies bind to the tumor cell membrane, activate complement, and lyse the tumor cell. An alternative mechanism of tumor cell lysis by antibody is **antibody-dependent cell-mediated cytotoxicity (ADCC).** In this mechanism, antibodies (usually of the IgG class) form an intercellular bridge by binding to a specific determinant on the target cell and the Fc receptor of effector cells (macrophages, granulocytes). ADCC is a more efficient in vitro lytic mechanism than complement-mediated cytotoxicity, requiring fewer antibody molecules per cell to kill. Comparisons of the efficacy of serotherapy in normal and complement-deficient tumor-bearing hosts have suggested that complement-mediated lysis may not be an important in vivo mechanism for killing tumor cells. Studies using monoclonal antibodies of different isotypes have also suggested that ADCC may be the more important in vivo effector mechanism. For these experiments, class-switch variants of monoclonal antibodies to tumor-associated antigens were selected in vitro, so that a panel of antibodies with the same tumor antigen specificity but different immunoglobulin isotypes was available. The antitumor activity of these antibodies following infusion into tumor-bearing hosts correlated with the efficiency of the isotype in mediating ADCC and not complement-dependent lysis.

Natural Killer (NK) Cells

A potential role for NK cells in tumor immunity has been suggested both by the ability of NK cells to kill a wide range of tumor targets in vitro and by the correlation between the level of host NK activity and resistance to the growth of transplantable tumor cells. The mechanism by which NK cells preferentially recognize and lyse transformed but not normal targets is unclear. This recognition does not appear to require binding to an antigen-specific receptor. Some studies have suggested that particular carbohydrate moieties may be expressed in increased density on the surface of NK-sensitive tumor cells and may function as a lectin that binds to and triggers NK cells. Cytolysis by NK cells appears to be mediated by the release of a cytotoxic factor.

The cytotoxic activity of NK cells can be augmented both in vitro and in vivo by the lymphokines IL-2 and interferon. Thus, NK activity can be amplified by immune T cell responses. Recent studies have demonstrated that augmentation of NK activity in visceral organs enhances resistance to the growth of metastases. NK cells represent a first line of host defense against the growth of transformed cells at both the primary and metastatic sites, as well as an effector mechanism that can be recruited by T cells to supplement specific antitumor responses.

Additional cytotoxic effector cells, which can be distinguished from classic NK cells, have also been identified. Natural cytotoxic cells appear to kill a spectrum of tumor targets different from NK cells. In contrast to NK cells, natural cytotoxic cells are resistant to glucocorticoids. **Lymphokine-activated killer (LAK) cells** can be induced by very high doses of IL-2. They are phenotypically distinct from NK cells and kill a much broader spectrum of tumor targets. The role of these effector cells during physiologic antitumor responses remains to be elucidated, and it is unclear if these represent cell types truly distinct from NK cells, or cells of the same lineage at different stages of activation and differentiation.

Macrophages

A substantial amount of data suggest that macrophages are important in tumor immunity not only as antigen-presenting cells but also as potential effector cells mediating tumor lysis. Resting macrophages are not cytolytic to tumor cells in vitro but can become cytolytic if activated with a **macrophage-activating factor (MAF).** MAF is commonly secreted as a lymphokine by T cells following antigen-specific stimulation, and thus participation of macrophages in the antitumor response may be dependent upon T cell immunity. The only currently well-defined MAF capable of rendering macrophages tumoricidal is IFN γ, but several recent studies using products of cloned T cell lines have suggested that other lymphokines with this activity may exist.

Analysis of the cells surrounding tumors has provided supportive evidence for the role of macrophages in immune-mediated tumor rejection. Macrophages isolated from tumors undergoing regression exhibit tumoricidal activity, whereas macrophages isolated from progressing tumors have generally shown no evidence of cytotoxic activity. Studies in which tumor cells and potential effector cells are implanted in hosts

in diffusion chambers to permit control and analysis of cell interactions have confirmed that macrophages can be activated by immune T cells to kill tumor cells in vivo. These same studies have demonstrated that tumor lysis is dependent upon intimate macrophage-tumor contact, although the precise mechanisms by which activated macrophages recognize and lyse tumor cells have not been defined. Both Th and Tc secrete MAF in response to antigenic stimulation, and the activation of macrophages to kill tumor cells may be important for the antitumor effects of each of these subsets. In the case of class II-restricted Th that cannot directly recognize most tumor cells, activation of an effector cell such as tumoricidal macrophages would be necessary to mediate an antitumor effect. In the case of Tc, activation of macrophages that can kill transformed cells may be an ideal supplementary lytic mechanism for eliminating potential variants in the tumor mass which may lose expression of the immunogenic determinant being recognized by T cells.

POTENTIAL MECHANISMS BY WHICH TUMOR CELLS MAY ESCAPE FROM AN IMMUNE RESPONSE

The concept of host **immunologic surveillance,** with the immune system providing the function of surveying the body to recognize and destroy frequently developing immunogenic tumor cells, was formally proposed by Burnet. This theory has been difficult to substantiate with experimental data. The high frequency of tumor development in immunosuppressed hosts was the major evidence initially supporting this hypothesis. More careful analysis revealed that there was not a general increase in all tumors but that tumors of lymphoreticular origin predominated. The failure to demonstrate a generalized increased appearance of immunogenic tumors in immunodeficient hosts has modified current views of immunologic surveillance. Effector populations such as NK cells rather than tumor antigen-specific immune responses are now believed to be important in the rejection of newly appearing transformed cells. However, it should be emphasized that antigen-specific responses do appear to provide a surveillance function to prevent the development of selected tumors, such as those induced by oncogenic DNA and RNA tumor viruses. The failure of the immune system to prevent the emergence of new tumors does not mean that tumor-specific immune responses do not develop during the growth of established tumors. Unfortunately, this response, which may be detectable during early but not late tumor growth, is usually ineffective. One important goal in tumor immunology is to determine why this response is ineffective and the mechanisms by which tumor cells circumvent or suppress a potentially effective immune response.

Selection of Less Immunogenic or Antigen-Negative Variants

Analysis of the tumor cells present in a tumor mass often reveals a remarkable degree of heterogeneity with respect to morphology and surface phenotype. Although many of these disparities are cell cycle-dependent, cloning of tumor cells has frequently revealed distinct heritable phenotypes, some of which are characterized by diminished expression of the tumor antigen recognized by the immune system. These cells will have a selective growth advantage. As growth of this spontaneous tumor variant proceeds, it will become increasingly difficult to identify that a host response to the tumor has been generated. One experimental example of this may be tumors induced with the chemical carcinogen MCA, in which rapidly appearing tumors tend to be highly immunogenic, whereas tumors that take a long time to appear tend to be nonimmunogenic, presumably owing to selection with elimination of the immunogenic clones.

Antigenic Modulation

This phenomenon is similar to the spontaneous generation of weakly immunogenic tumors described above. However, here the immune response to a tumor antigen induces the growth of antigen-negative cells. This loss of antigen expression reflects only a phenotypic change in the tumor cell, and if the immune response is ablated, the antigen will be reexpressed. Antigenic modulation resulting from antibody responses has been extensively reported, but modulation as a consequence of a T cell response has not yet been clearly identified. Antigen loss appears to result from capping of the antigen followed by endocytosis or shedding. The initial description of this phenomenon was in murine leukemias expressing the thymic lymphocyte (TL) antigen, but similar observations have been made with human B cell tumors and leukemias.

Blocking Factors

Serum of hosts bearing progressive tumors has been shown to specifically inhibit both cell and antibody-mediated cytotoxicity to the tumor. The precise nature of the factors in serum that block immune function remains controversial, and it is likely that there are many different types of blocking factors. For example, noncytolytic antibody in the serum may bind to the relevant target antigen and thereby interfere with recognition by cytolytic antibodies or effector T cells. Antigen-antibody complexes may occupy Fc receptors on cells mediating ADCC and block their effective function. Some of the blocking factors may actually be suppressor factors released from suppressor T cells and thus may specifically down-regulate the immune response.

Nonspecific Suppression

There are many mechanisms by which tumor cells can nonspecifically interfere with the expression of immunity in the host. Some tumor cells can be shown to release soluble factors that directly suppress immunologic reactivity. Protein-calorie malnutrition commonly associated with progressive tumor growth

can cause a generalized decrease in cellular and humoral immunity. Perhaps the best-studied phenomenon is the inhibition of immune responses by macrophages obtained from hosts bearing progressive tumors. This appears to be largely mediated via the secretion of prostaglandins, and in vitro treatment of macrophages with the cyclooxygenase inhibitor indomethacin can overcome the inhibitory effects. However, there are at present few data to suggest that in vivo treatment with indomethacin will augment the depressed immunologic reactivity of hosts with advanced cancer.

Specific Suppressor Cells

The presence of suppressor T cells (Ts) that specifically inhibit immune reactivity to a tumor may represent a major reason for the difficulties in detecting tumor-specific immunity in cancer patients. For example, even with many of the highly immunogenic animal tumors studied in the laboratory, tumor-specific immunity cannot be identified in hosts bearing progressive tumors owing to the presence of Ts. Analysis of a phenomenon called **concomitant immunity** has been very helpful for elucidating the evolution of host effector and suppressor responses to a progressing immunogenic tumor. For these studies, a lethal dose of an immunogenic syngeneic tumor is inoculated into the host. One week later when a growing tumor mass is evident, the animal is challenged with a smaller dose of the same tumor at a second site. This secondary tumor challenge will usually be rejected while the primary tumor continues to grow, implying that an effector response has been generated in the host which is adequate for a small tumor mass but which developed too late to keep pace with a large, rapidly proliferating tumor mass. If challenge with the small dose of tumor is delayed for another week until the primary tumor has grown larger, the secondary tumor challenge will grow progressively and will not be rejected. The loss of concomitant immunity in this setting can be shown to result from the development of Ts that interfere with the expression of T cell immunity. Thus, an initially demonstrable tumor-specific T cell response to a growing tumor can become undetectable as the tumor progresses because of the development of Ts, and studies performed after significant tumor progression may give the false impression that no immune response has been generated.

IMMUNOTHERAPY

Although the host response to a tumor may be inadequate for controlling tumor growth, the identification of immunogenic determinants on many tumor cells and an improved understanding of the mechanisms by which tumors evade immunity suggest that it may be possible to manipulate or amplify the immune response to promote tumor eradication. Several distinct approaches to such immunotherapy are now being studied.

Immunization With Tumor Cells or Purified Antigens

Immunization of hosts bearing established progressing tumors with tumor cells or tumor antigen has generally been ineffective in causing tumor regression. It is now evident that such an approach is doomed to failure even with immunogenic tumors, since the tumor is likely to have elicited a Ts response that inhibits tumor reactivity. Therefore, prior to attempting sensitization, it is mandatory that the tumor burden be decreased or the host depleted of Ts. Experimental models to examine the potential utility of tumor immunization have now been developed. Hosts bearing an advanced primary tumor and micrometastatic lesions undergo resection of the primary tumor mass to reduce the tumor burden. Since Ts induced by the large tumor should have a short life span after resection of the tumor mass, the hosts are not treated for a brief period to allow for decay of the Ts. Immunization of these hosts with killed tumor cells or purified tumor antigens has been shown to have a significant therapeutic effect on the outgrowth of the residual micrometastatic tumor. Thus, in the proper setting, it may be possible to augment immunity to an established tumor. Approaches similar to this are being studied in patients with colon cancer, with some preliminary evidence that a potentially beneficial antitumor response is being elicited.

Since many tumors are only weakly immunogenic, several methods have been used to augment the response to the tumor determinants. Initial attempts utilized neuraminidase to alter the tumor cell surface and potentially unmask new tumor determinants. This has had only limited success. More recently, modification of the tumor cell by binding a hapten such as trinitrophenyl (TNP) or by infection with a virus such as vaccinia has yielded promising results. Animals immunized with TNP- or virus-modified tumor cells generated an augmented specific antitumor response, presumably because of the generation of a large number of helper T cells to the new, immunogenic determinants on the tumor cell that can amplify the tumor-specific response to the same cell. Further studies with this approach may provide valuable insights into how the response to weakly immunogenic tumor determinants can be amplified.

Adoptive Cellular Immunotherapy

Animal models have been developed in which hosts bearing advanced tumors can be treated by the transfer of tumor-specific syngeneic T cells. These models using syngeneic donor T cells immune to the tumor have served as prototypes of what might be achievable if the host immune response to an autochthonous tumor could be selectively amplified. A major obstacle to this adoptive therapy is the presence of Ts in the tumor-bearing host that interfere with the expression of transferred immunity. It is usually necessary to eliminate these Ts, either with nonspecific cytotoxic reagents (such as cyclophosphamide) or with specific reagents that deplete all host T cells.

Complete tumor elimination following adoptive therapy requires a prolonged time period, and the cells transferred must therefore be capable of persisting in the host in order to be effective. Noncytolytic lymphokine-producing Th as well as Tc have been shown to mediate antitumor effects in these models, confirming that additional immunologic effector mechanisms recruited by Th can make important contributions to tumor eradication in vivo.

Application of these studies in animal adoptive therapy models to the treatment of human cancers will require isolating the presumably small number of tumor-reactive lymphocytes present in the patient, expanding these cells to large numbers in vitro, and then reinfusing the cells into a host that has been depleted of Ts. This might be achievable by stimulating unfractionated patient lymphocytes in vitro with tumor antigen, inducing the small number of tumor-reactive T cells to proliferate with IL-2, and then expanding these cells by repeated culture with tumor antigen and IL-2. Studies in animal models have confirmed that tumor-specific T cells can be expanded to large numbers in vitro with these methods and then used in specific adoptive therapy of disseminated tumors. Since these cultured T cells expanded in the presence of exogenous IL-2 are dependent upon IL-2 in vitro for survival, the efficacy of infusing exogenous IL-2 after cell transfer was examined. These studies showed that the in vivo administration of exogenous IL-2 induced the in vivo proliferation, prolonged the survival, and augmented the therapeutic efficacy of cultured T cells. Thus, infusion of exogenous IL-2 may prove to be a useful adjunct to specific adoptive therapy of in vitro expanded human tumor-reactive T cells.

During examination of the in vitro effects of increasing doses of IL-2 on the generation of tumor-specific T cells, it was observed that a cytolytic effector cell lacking antigen specificity but displaying a marked preference for transformed cells was induced. These lymphokine-activated killer (LAK) cells, generated only in the presence of exceptionally high nonphysiologic doses of IL-2, have now been extensively characterized and studied both in vitro and in vivo. Administration of cytolytic LAK cells, particularly in association with IL-2, has been shown to mediate a significant in vivo antitumor effect in several animal models and, more recently, has shown substantial activity in the treatment of human tumors. Although the efficacy of treatment with LAK cells and IL-2 appears to be limited by a lack of absolute specificity and some toxicity, there are many clinical settings, such as isolated pulmonary or liver metastases, in which it may be possible to utilize these effector cells to achieve a directed antitumor effect.

Administration of Monoclonal Antibodies

The development of the technology for generating monoclonal antibodies has converted the previously unpromising field of tumor **serotherapy** into a form of treatment with enormous potential. Despite occasional reports of exciting clinical results, a great number of biologic problems still need to be overcome for this modality to be generally effective. Studies in animal models with tumor-specific antibodies have demonstrated that even enormous titers of antibody can eliminate only relatively small tumor masses. Antibodies do not appear to effectively penetrate large tumor masses, and tumor escape mechanisms such as modulation of the target antigen from the tumor cell surface and selection of antigen loss variants appear to be relatively common. ADCC rather than complement-mediated cytotoxicity appears to be the major in vivo effector mechanism following infusion of unmodified antibody, and thus IgG2a antibodies have tended to be the most effective.

Several approaches are being studied to augment the therapeutic activity of monoclonal antibodies. The most promising involve conjugation of cytotoxic drugs, toxins, or radioisotopes to the antibody to deliver a lethal hit directly to the tumor without requiring participation of host effector cells. The use of radioisotopes that kill by emitting ionizing radiation may be particularly useful, since it may be possible to kill antigen-negative tumor variants in the tumor mass if they are in the proximity of antibody-binding tumor cells.

Most of the tumor-reactive monoclonal antibodies recognize tumor-associated rather than tumor-specific antigens and thus are likely to recognize some normal tissues. Consequently, administration of some antibodies or antibody conjugates may prove to be unacceptably toxic to the host. Future studies will need to carefully define the distribution of normal antigens recognized by each antibody to be used in therapy. It seems safe to predict that sufficient numbers of monoclonal antibodies will be identified to permit treatment of a wide variety of human tumors.

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Immediate Hypersensitivity

15

Oscar L. Frick, MD, PhD

The term immediate hypersensitivity denotes an immunologic sensitivity to antigens that manifests itself by tissue reactions occurring within minutes after the antigen combines with its appropriate antibody. Such a reaction may occur in any member of a species (anaphylaxis) or only in certain predisposed or hyperreactive members (atopy).

In 1890, von Behring discovered the prophylactic use of antiserum against diphtheria toxin. In the search for other prophylactic antisera, Portier and Richet noted an immediate shocklike reaction in a sensitive dog to a sea anemone toxin; this harmful reaction they called **antiphylaxis** (or anaphylaxis), to distinguish it from the helpful **prophylaxis.** Within the next decade, hay fever and asthma were recognized as human counterparts of animal anaphylactic reactions, and histamine was considered to be the primary pharmacologic mediator of such symptoms. Later it was discovered that skin tests could be used for the specific diagnosis of the troublesome antigen and that a prolonged series of injections of these antigens could help relieve allergic symptoms.

ANAPHYLAXIS

Anaphylaxis is a manifestation of immediate hypersensitivity resulting from the in vivo interaction of cellular sites with antigen and specific antibody.

Generalized anaphylaxis is a shocklike state occurring within minutes following an appropriate antigen-antibody reaction. Upon the first exposure of an animal to an antigen, a cytotropic antibody can form that sensitizes mast cells and basophils in tissues and blood, respectively. After a second exposure to the antigen, the sensitized animal reacts to histamine and other mediators released by mast cells as a result of the antigen-antibody reaction. Histamine causes a marked vasodilatation and leakage of intravascular fluids, resulting in shock. There are smooth muscle spasms, especially in certain smooth muscle-containing organs such as guinea pig bronchi and canine liver "vessels." These are the principal specific shock organs in these 2 species. The extreme bronchoconstriction in the guinea pig results in respiratory obstruction, asphyxia, and death. The extreme vasodilatation and leakage of fluids in the dog or in humans causes profound shock and death. Epinephrine may be a lifesaving treatment in anaphylaxis.

Local anaphylaxis may occur in specific target organs such as the gastrointestinal tract, nasal mucosa, or skin. Experimentally, the skin or other tissue may be passively sensitized with serum from a sensitized animal; subsequent intravenous or local injection of antigen will result in a local anaphylactic reaction. This method has been used for the passive cutaneous anaphylaxis (PCA) test, in which the skin of an animal (guinea pig, rabbit, human, rat or mouse, etc) is injected with serum from a sensitive animal of the same or another species. After an appropriate latent period, the antigen is given intravenously along with Evans blue dye, and this will react with the skin-fixed antibody, causing the release of histamine. This results in vasodilatation and leakage of albumin, to which the blue dye is attached, producing a blue spot. This blue spot indicates that an anaphylactic reaction has taken place in the skin.

In Vitro Anaphylaxis

Several tissue models of anaphylaxis have been developed for experimental studies. The Schultz-Dale test uses isolated smooth muscle from a sensitized animal. Ileum, uterus, or a tracheal ring is suspended in physiologic buffered Tyrode's solution. Such smooth muscle strips can also be passively sensitized by bathing them in serum from a hypersensitive animal. After addition of antigen to the bath, smooth muscle contractions occur within seconds or minutes. Alternatively, finely chopped lungs or skin fragments from actively sensitized animals (or such fragments passively sensitized with serum from an immunized animal) are suspended in physiologic buffer. Addition of antigen causes release of histamine and slow-reacting substance of anaphylaxis (SRS-A), which is quantitated by bioassay or chemical means. Peritoneal mast cells from actively or passively sensitized rats, upon addition of antigen, show visible degranulation, and histamine and serotonin release may be measured in the supernate.

Any antigen should be able to elicit anaphylaxis in a properly sensitized animal. These may be proteins, chemical haptens such as drugs attached to proteins, carbohydrates, or, occasionally, nucleic acids. These antigens usually must be soluble antigens. Cellular antigens such as sheep red cells or bacteria cause weak anaphylaxis, which probably indicates that soluble antigens are eluted from the cell surface to participate in such reactions.

Antibody Classes

Cytotropic antibodies, especially of the IgG and IgE classes, are involved in anaphylactic sensitization. In special circumstances, IgA and IgM may also be involved. Homocytotropic antibodies—antibodies that will sensitize an animal of the same species—are usually considered cardinal in such reactions. These are IgE in all species so far examined, γG_1 or γG_a electrophoretic mobility in guinea pigs and rats, respectively, and IgG4 in humans. Heterocytotropic IgG antibodies and occasionally IgM and IgA antibodies can passively sensitize tissues (especially skin) of other species. The classic example is rabbit IgG antibody, which can passively sensitize guinea pig skin for PCA. On the contrary, IgG antibodies of ungulates such as sheep, goats, and horses are unable to sensitize guinea pig skin for PCA. Apparently, the former have a specific skin-fixing site on their Fc portions.

Sensitization of Target Tissue for Anaphylaxis

In most cases, 10–14 days are required after immunization before IgG antibodies result in active anaphylactic sensitization; IgE sensitization may occur somewhat earlier. Passive sensitization has a latent period (time between injection of antibody and challenge with antigen) of 3–6 hours for IgG antibodies. This latent period allows antibody to "fix" to the target mast cells. However, the IgG passive sensitization is short-lived; the IgG molecules become detached within 12–24 hours. An antigenic challenge at this later time causes no (or minimal) anaphylaxis. There is an inverse squared relationship between the local IgG antibody concentration (c) and time (t) of the latent period: $c = 1/t^2$. In fact, if there is sufficient antibody concentration, the latent period can be reduced to almost zero and the reaction can occur immediately after passive sensitization.

Passive sensitization with IgE antibody may occur within 6 hours but becomes stronger by 24–72 hours, the usual time at which antigenic challenge is made. Anaphylactic reactions occurring after 48–72 hours are almost exclusively due to IgE antibodies (IgG antibodies are already detached and do not participate in such a reaction). IgE antibodies remain fixed to the target mast cells for many weeks, eg, 3 weeks in rats and 6 weeks in humans.

Molecular Models of Anaphylaxis

Following the first exposure to antigen, the animal responds with antibody formation (Fig 15–1). Cytotropic antibodies such as IgG or IgE fix to their respective receptors on the surface of mast cells, and the animal is considered sensitized. Such antibodies may also be passively introduced into the general circulation or into local tissues.

With the second exposure, the antigen seeks out these tissue-fixed antibodies and reacts with them at the cell surface (Fig 15–2). An antigen molecule combines with 2 antibody molecules to form a bridge. With Ab molecules anchored to receptors, this bridging brings together 2 IgE receptor molecules, which triggers an enzymatic cascade at the cell surface that causes the dissolution or expulsion of mast cell granules. These granules dissolve and release histamine, serotonin, and heparin, their pharmacologically active agents. Free histamine and serotonin act on adjacent smooth muscle and vascular endothelial cells, causing

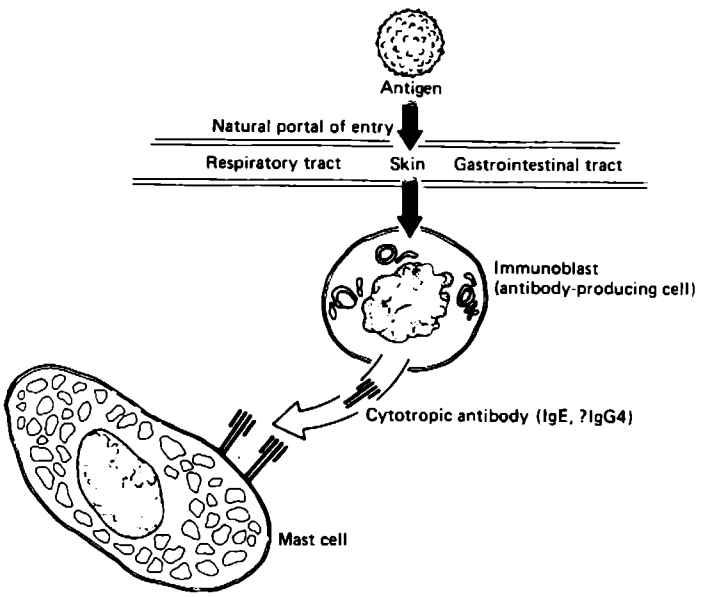

Figure 15–1. Atopic sensitization.

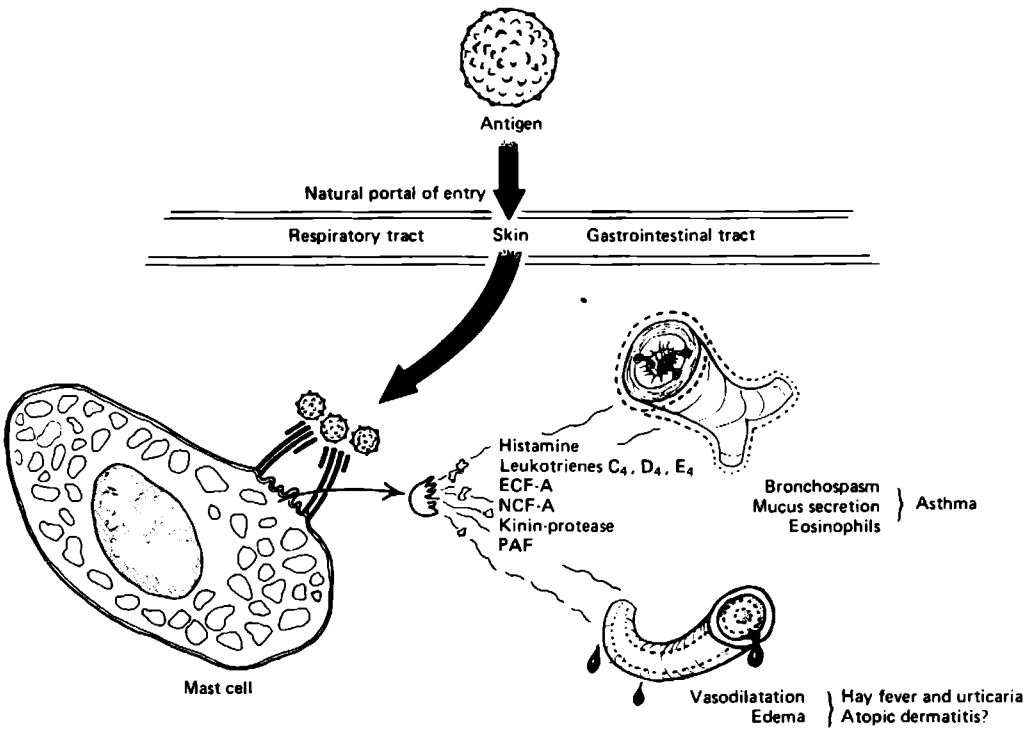

Figure 15–2. Atopic reaction.

the clinical symptoms of anaphylaxis (eg, bronchospasm and edema). Other mediators are also released from mast cell granules—leukotrienes, eosinophil and neutrophil chemotactic factors of anaphylaxis (ECF-A, NCF-A), and kinin-generating protease. These also exert their pharmacologic effects on neighboring cells. The optimal ratio of antigen to antibody to elicit anaphylaxis is slight to moderate antigen excess. So-called toxic complexes of composition Ag_3Ab_2 or Ag_5Ab_3 best trigger such anaphylactic reactions. These are small complexes which best bridge surface receptors in the combining site of sensitizing antibodies. Complexes of Ag_2Ab_1 formed in extreme antigen excess do not trigger anaphylaxis. Such complexes are unable to bridge antibodies because both combining sites on each antibody molecule are attached to 2 different antigen molecules. Large amounts of antibody are complexed by relatively few antigens in zones of antibody excess or at the optimal proportions for precipitation. These are usually insufficient for triggering anaphylactic reactions, but in these zones minimal anaphylaxis can occur.

This molecular model of anaphylaxis can be duplicated with immunoglobulin reacting with antibody molecules fixed to the mast cell. The reaction forms complexes of a composition appropriate to trigger anaphylaxis. Anti-IgE antibodies will complex with the Fc portion of the tissue-fixed IgE molecule, and such bridging will trigger histamine release. Similarly, tissue-fixed antibodies may be aggregated by mild heating or bisdiazotized benzidine. This creates nonantigenic bridges among tissue-fixed cytotropic antibodies and triggers the anaphylactic reaction. IgE or IgG antibody-fixing cell receptors on mast cells may be blocked with normal or myeloma proteins of the same class that compete with antibody molecules for these receptors. Such blocking of receptors by normal or myeloma proteins prevents antibody fixation, and no triggering of anaphylaxis is possible. Antireceptor antibodies bridge receptors directly to cause mediator release.

Target Cells & Mediator Release

Target cells for anaphylaxis are the tissue-fixed mast cells, especially in shock organs such as lung, bronchial smooth muscle, and vascular endothelium. Blood basophils may also act as target cells. Antibody molecules fixed to mast cells are physically distorted by antigen, and this activates several enzyme pathways similar to the classic complement cascade (see Chapter 10). The speed and completeness of activation of these enzyme cascades are modulated by the cAMP and calcium "second messengers" in the cytoplasm of such target cells. These cascades are energy- and calcium-dependent and cause dissolution or expulsion of mast cell granules. Positively charged histamine and serotonin are electrostatically complexed with negatively charged heparin-proteoglycan.

After dissolution of the granule, positively charged sodium ions from extracellular fluid exchange with positively charged histamine and serotonin on heparin and cause their release in a free state. Free histamine and serotonin exert their pharmacologic effects on adjacent smooth muscles and vascular endothelial cells. Similar mechanisms probably exist for the release of other preformed mediators: ECF-A, NCF-A, and proteases. Cromolyn sodium apparently can stabilize mast cell membranes or granules and can prevent the release of mediators.

In summary, anaphylaxis is a reaction found in almost all vertebrate animal species (hamster excepted) that results from sensitization of tissue-fixed mast cells by cytotropic antibodies following exposure to antigen. Subsequent exposure to antigen results in complexing of an antigen molecule with 2 antibody molecules in mild to moderate antigen excess. Complexed antibody molecules are physically distorted and initiate enzyme cascades ending in the release of pharmacologic mediators that exert their effects upon adjacent target tissue.

ALLERGY & ATOPY

Von Pirquet proposed the term **allergie** (Gk *allos* "other" + *ergon* "work") in 1906 to denote an immune deviation from the original state or a "changed reactivity" of an individual. An allergic individual was one who deviated from the expected immunologic response. Von Pirquet included all forms of altered immunologic responsiveness, encompassing reactions to toxins, bacteria, and other infectious agents; pollen hay fever; and urticaria produced by foods. Coca in 1923 coined the word **atopy** (Gk *atopos* "uncommon") to denote an abnormal state of hypersensitivity as distinguished from hypersensitivity responses in normal individuals, eg, contact dermatitis, serum sickness, anaphylaxis, and infection with tubercle bacilli.

Clinical Types of Atopy

Until recently, atopy was thought to be restricted to humans, but such conditions have now been described in dogs, rats, and even a baby walrus. In genetically susceptible individuals, atopy may affect one or more primary shock organ systems. In humans, atopy involving the respiratory system (the nasal mucosa, bronchioles, and aural mucosa) can cause hay fever, asthma, and serous otitis. With the skin as shock organ, urticaria, angioneurotic edema, and atopic dermatitis (eczema) occur. Sensitized intestinal and urinary systems react with antigen to cause vomiting, abdominal pain, diarrhea, and urinary frequency and pain on urination. Vascular involvement, especially of the central nervous system, may result in headaches, personality changes, and other nervous system manifestations.

Genetic Basis of Atopy

Hay fever and asthma can be familial. If both parents have atopy, there is a 75% chance of the child having atopic symptoms; with one parent involved, there is a 50% chance. Thirty-eight percent of atopic patients have no parental history of atopy.

With the recent discovery of immune response genes associated with HLA haplotypes, a ragweed hay fever haplotype has been postulated. Ragweed-allergic patients in a family tree had the same HLA haplotype; whereas nonallergic members of the family had different haplotypes. The numerical haplotype designation among allergic families varied widely, but within a family, ragweed sensitivity was associated with a single haplotype.

In large Caucasian population samples, there appear to be HLA associations with certain ragweed and grass pollen fractions (Table 15–1). It is suggested that genetic factors control the immune response and perhaps also the pharmacologic regulation and pathologic expression of allergy. The best studies have been done on genetic control of immune response, where 2 antigen-specific factors, Ir and Is genes, are linked to HLA loci or controlled by genes associated with the HLA complex. Hyperresponsiveness to particular antigens (cited above) appears associated with alleles of the 2 most common HLA haplotypes. Furthermore, regulation of total IgE concentration is not HLA-linked. Hyperresponsiveness of airways to methacholine has a familial aspect. Much more work is required to establish the genetic components in atopy. Blumenthal and others found that identical twins whether raised together or in different environments had nearly identical IgE antibodies to ragweed and other pollens, which indicates a strong genetic factor in allergic responsiveness to particular antigens.

Allergens

Allergens are the antigens that give rise to allergic sensitization of the IgE antibody class. Most natural allergens are somewhat peculiar in that their molecular weight appears to be restricted to the range 10,000–70,000. Smaller molecular antigens, unless polymerized, would be unable to bridge adjacent IgE molecules and receptors. Large allergens (MW > 70,000) might not penetrate mucosal surfaces sufficiently to reach IgE antibodies on cell receptors.

Table 15–1. Associations of pollen sensitivity with HLA types.

Pollen Antigen	HLA Association*	
	Positive	Negative
Ra5	D2, B7	
Ra3	A2, A28	A3, A11
AγE	0	0
Rye I	B8, D3, A1	A9
Rye II	D3, B8	
Timothy A3	B7	A9, A10

*A positive association indicates a significant association between positive skin tests or the radioallergosorbent test (RAST) and a given HLA type. A negative association indicates a statistically significant relationship between the absence of positive skin test or RAST reactivity and a given HLA type.

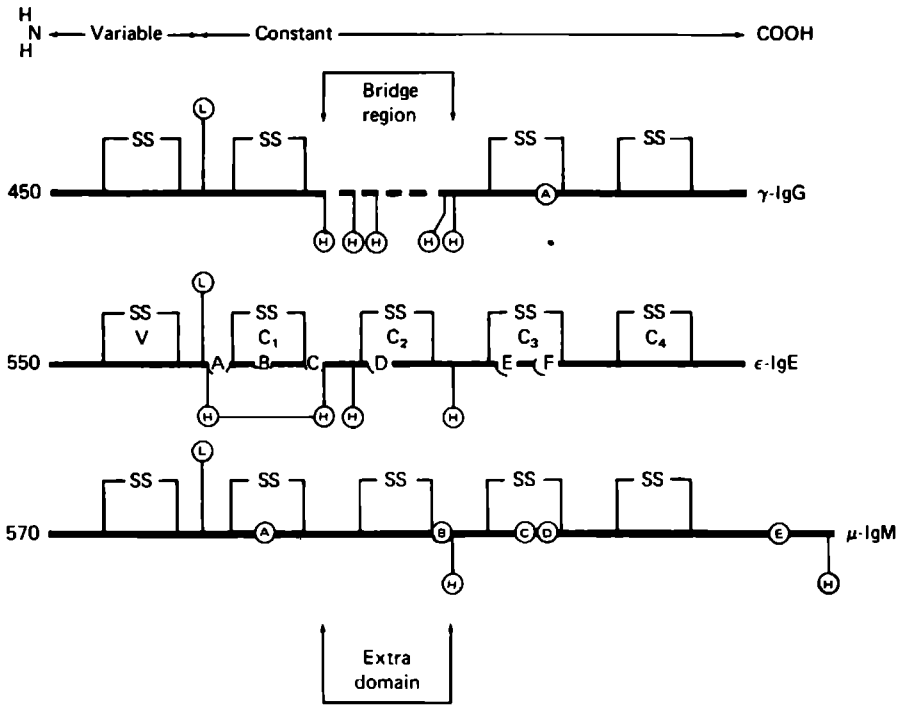

Figure 15–3. Diagrammatic representation of domains, inter-heavy chain bonds (L = light chain, H = heavy chain), and carbohydrate units (A,B,C, etc). Deletion of a counterpart to the $C_\epsilon 2$ and $C_\mu 2$ domains is one possible explanation of the general structure of the bridge region in the γ chain. (Reproduced, with permission, from Bennich H: Structure of IgE. *Prog Immunol* 1974;2:49.)

These large allergens are extremely polar compounds, induce sensitization in very small amounts (nanograms to micrograms), and have many sulfhydryl groups, indicating much cross-linking.

An allergen can result in both IgE and IgG antibodies, but mild formalin or glutaraldehyde treatment to form "allergoids" reduces the allergenicity (IgE formation) without affecting the antigenicity (IgG "blocking" antibody formation). Such polymerized pollen antigens greatly enhance IgG blocking antibody production, with little change in IgE antibodies and good clinical improvement, so that frequency of injections can be markedly reduced.

Antibodies

Prausnitz and Küstner used serum to passively transfer the allergic reactivity from an atopic patient to the skin of a normal individual. These antibodies were subsequently called reaginic, or skin-sensitizing, antibody. This reaginic antibody in humans has been identified as IgE. IgE has a molecular weight of 196,000 and a sedimentation coefficient of 8S; it is a fast γ_1-globulin on electrophoresis, has a high carbohydrate content of 12%, and consists of 2 light chains (either type κ or λ) and 2 heavy chains of type ϵ.

The 550-amino-acid sequence of the ϵ heavy chain of myeloma IgE ND* has been established and is similar to the μ chain of IgM except for the C-terminal 19 amino acids (Fig 15–3). The ϵ heavy chain consists of one V region and four C domains, $C_\epsilon 1$, $C_\epsilon 2$, $C_\epsilon 3$, and $C_\epsilon 4$. There are 15 half-cysteines, 10 of which form one intrachain disulfide bond in each of the 5 domains. One interchain cysteine binds the light and ϵ heavy chains, and 2 inter-ϵ chain bonds occur just before and after the $C_\epsilon 2$ domain. There is a second intrachain disulfide bridge within the $C_\epsilon 1$ domain. Rich in carbohydrate (12%), IgE has 6 oligosaccharide side chains of unknown function: 3 in the $C_\epsilon 1$, one in the $C_\epsilon 2$, and 2 in the $C_\epsilon 3$ domains (A–F in Fig 15-3).

The skin-fixing, or mast cell cytotropic, activity resides in the $C_\epsilon 3$ or $C_\epsilon 4$ domains, and the well-known heat lability of reaginic skin fixation involves these 2 regions. Reduction of disulfide bonds also alters cytotropic activity. A working hypothesis is that firm attachment of the IgE molecule to the mast cell membrane involves at least 2 kinds of sites with the $C_\epsilon 3$ and $C_\epsilon 4$ regions of the ϵ chain. The primary recognition site for the mast cell surface receptor appears to be located in the $C_\epsilon 4$ region. Secondary binding may be located in either $C_\epsilon 3$ or $C_\epsilon 4$. And, finally, the inter-

*IgE ND = the IgE myeloma protein from "patient ND."

heavy chain bond assisting half-cysteine 318 may exert an avidity effect which binds the ϵ chain firmly to the mast cell membrane.

The primary biologic property of IgE is tissue fixation, ie, cytotropism for the mast cell and basophil membranes. Like IgA, IgE does not fix complement by the classic pathway. In very large amounts, IgE can fix C3 by the alternative pathway, but this is probably not biologically relevant. IgE does not cross the placenta. Nine patients with IgE myeloma have been discovered, and these have provided sufficient material for the above structural and sequencing data.

Animal antisera to human IgE myeloma proteins have been labeled with fluorescein or ^{125}I, and the labeled antisera used to localize the tissue and cellular distribution of IgE. Plasma cells forming IgE have been found extensively in the secretory surfaces inside the body, eg, the bronchi and bronchioles of the respiratory tract, the gastrointestinal mucosa, and the urinary bladder. The tonsils and adenoids were especially rich in IgE-forming plasma cells. This secretory distribution of IgE-forming cells is similar to that of secretory IgA-producing plasma cells. Thus, both can be considered secretory immunoglobulins.

The highest concentrations of IgE are found in nasal polyps, particularly in polyps of allergic individuals. The systemic lymphoid organs, such as the spleen, liver, and regional lymph nodes, have only rare IgE-forming lymphocytes (which is also true for the circulating blood). When radiolabeled IgE was injected into monkeys, IgE was found only on blood basophils and tissue mast cells. No other cells contained surface IgE. On electron microscopy, basophils are seen to have IgE in large patches on their surfaces. With high concentrations of antibody, capping occurs, and the cell eventually removes the cap. The number of IgE molecules on a basophil surface has been estimated at 5300–27,000 in nonallergic individuals and at 15,000–41,000 in highly allergic individuals. Passive sensitization with IgE myeloma protein of a normal individual's basophils (containing 5300 IgE molecules) raised the number of IgE molecules to 36,000—the same as in the highly allergic individual.

Measurement of IgE

There is so little IgE in the serum that it escaped detection for many decades because the methods for immunoglobulin detection were not sufficiently sensitive. The normal adult IgE level is about 250 ng/mL. In severely allergic individuals, where IgE concentration is about 700 ng/mL, a specific IgE precipitin band is detectable by Ouchterlony immunodiffusion. However, for most studies such sensitivity is not sufficient, and radioisotopic or enzyme-labeled methods are required for IgE quantitation.

A. Radioimmunosorbent Test (RIST) for Total IgE Concentration: This most sensitive of the detection methods detects 1 ng or less of IgE in serum. Rabbit anti-IgE myeloma protein is coupled to cyanogen bromide-activated filter paper disk (PRIST). Dilutions of an IgE-containing standard serum or an unknown patient's serum are reacted with the anti-IgE-coated particles. After thorough washing, the particles are reacted with ^{125}I rabbit anti-IgE myeloma protein. After additional washing, the particles are counted in a gamma counter to determine the amount of bound IgE. In many laboratories, enzymes such as β-galactosidase are replacing radioactive labels, converting them to enzyme-linked immunosorbent assays (ELISA).

B. Radioallergosorbent Test (RAST) for Specific IgE Concentration: Purified allergen extract is coupled to cellulose particles, paper disks, or microtiter plate wells. Patient's serum containing IgE antibody or a standard serum is reacted with the allergen-coupled immunosorbent. After thorough washing, ^{125}I-labeled rabbit anti-IgE is reacted with the immunosorbent (Fig 15–4). After further washing, the radioactivity on the centrifuged sorbent is determined and is a measure of the amount of specific serum IgE antibodies to that allergen. Enzyme or fluorescein substitution for radioisotope is becoming common prac-

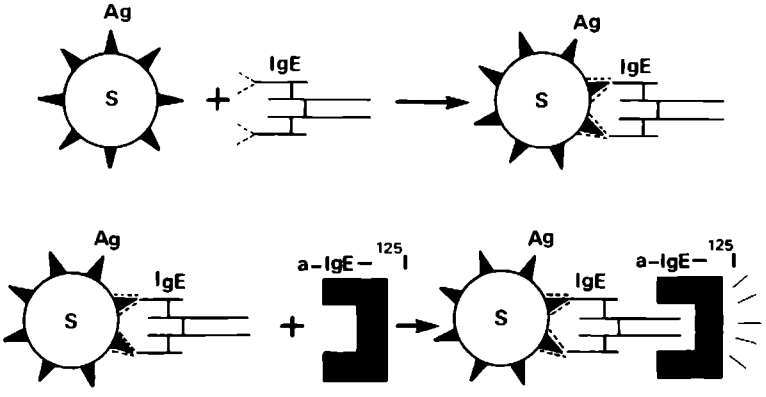

Figure 15–4. Schematic diagram of the radioallergosorbent test (RAST). S is the sorbent, with antigenic determinants (Ag). Human IgE attaches to the antigen and is detected with radiolabeled anti-IgE.

tice. These methods measure 1 ng of specific IgE antibody.

Specific IgE antibodies in human serum also have been measured by passively sensitizing monkey tissues, such as strips of monkey ileum or finely chopped monkey lung or skin tissues. The passively sensitized monkey smooth muscle preparation is suspended in a buffer bath, and allergen added to the bath will cause contractions of the smooth muscle that can be measured by a kymograph. Passively sensitized monkey lung or skin fragments are reacted with allergen and centrifuged. The supernate is then measured for histamine or leukotriene.

Serum Concentrations of IgE

Serum concentrations of IgE can be expressed in international units (IU): 2.3 ng IgE = 1 IU. The normal newborn has virtually no IgE in its cord serum (< 0.9 IU/mL). By the age of 1½–4½ months, the mean is 9 IU/mL in healthy children. This increases to 32 IU/mL between 9 months and 3 years of age. The adult level is about 90 IU/mL (with a range of 29–800 IU/mL).

In a group of allergic children, 17 out of 21 children with asthma had elevated serum IgE levels (Fig 15–5). Three of the remaining children had IgE levels in the upper normal range. Only 7 of 21 children with allergic rhinitis had increased IgE, while 14 were in the upper normal range. IgE levels present in nasal secretions mirror those in the serum. IgE levels in nasal secretions ranged from 10–150 ng/mL in a group of normal children and adults to 36–850 ng/mL in a group of asthmatic children. A group of Ethiopian children with active *Ascaris* infection were noted to have a mean serum IgE level of 4400 ng/mL (range, 240–14,300 ng/mL), which is 30 times the normal IgE level. Subsequently, patients with other parasitic infections, especially other roundworms, were found to have extremely high serum IgE levels. Patients with atopic dermatitis had an IgE level 9 times the normal mean, whereas patients with urticaria and other dermatoses had normal IgE levels.

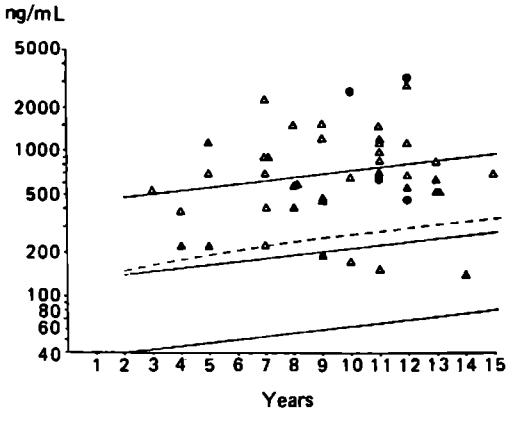

Figure 15–5. Serum IgE concentrations in children with asthma (△), asthma plus eczema (▲), and allergic rhinitis (hay fever) during the pollination season (●). The solid lines (–) represent the regression line and 95% confidence limits calculated on the logarithmic IgE values in 132 healthy children; the dotted line (---) represents the regression line calculated on the arithmetic IgE values in the same children. (Reproduced, with permission, from Berg T, Johansson SGO: IgE concentrations in children with atopic diseases. *Int Arch Allergy Appl Immunol* 1969, 36:220.)

Measurement of total IgE levels may be useful in the early detection of allergy in infants. Two European studies showed that a cord serum IgE level greater than 0.9 IU/mL was a good predictor of the subsequent development of atopy. A survey in the USA of 34 infants from allergic families found 11 to have serum IgE levels greater than 20 IU/mL at age 12 months. Ten of these 11 children had allergic symptoms at age 2.

The serum IgE level apparently reflects an excess of IgE antibody in a pathophysiologic sense, because allergic reactions occur upon sensitized mast cells in tissues. The amount of IgE in skin tissue can be measured by injecting dilute rabbit anti-IgE serum into the skin. Normal serum IgE concentrations of 65–130 IU/mL gave a threshold skin reaction with a 1:10,000 dilution of rabbit anti-IgE serum. In highly allergic patients, a 1:20 million dilution of rabbit anti-IgE still reacted in the skin. There was a rough correlation between minimal tissue concentration of IgE necessary to give a threshold skin response and the serum concentration of IgE. Measurement of skin IgE in this way is not generally recommended, because injecting a foreign animal serum into the skin could sensitize the patient to animal proteins.

Specific IgE Antibodies

The most widely used in vitro method of measuring specific IgE antibodies to a variety of allergens is the radioallergosorbent test (RAST) or enzyme modifications. This assay is used extensively for the diagnostic quantitation of IgE antibodies to a variety of allergens. It is used to measure IgE antibody titers during the treatment of patients and to standardize allergens in a modified test.

A group of 20 children with ragweed hay fever were followed for 5 years and treated with preseasonal ragweed injections for 4 of those 5 years (Table 15–2). Over the 4 years, IgE antibodies against ragweed fell and reached a low plateau. Concomitantly, the symptom index of hay fever fell and reached a plateau. IgG blocking antibodies rose during the therapy. Because the children were doing so well, with minimal symptoms, the preseasonal therapy was omitted in the fifth year. Subsequently, IgE antibodies rose, the symptoms became more severe, and the IgG blocking antibodies fell. This suggested that treatment had been stopped too soon and should be resumed. The radioallergosorbent test will probably find its greatest usefulness in monitoring the course of allergic therapy.

Using a standard allergen coupled to an immuno-

Table 15–2. Changes in symptoms and antibody levels produced by allergy treatment.*

Season	Number of Patients†	Mean Seasonal Symptom Index (SI)	Mean "Postseason" Allergen-Specific IgE Antibody Titer (units)	Mean IgG "Blocking Antibodies" (units)
1967 (control)	8	0.445	2,520	< 10
1967 (treated)	9	0.150	1,150	121
1968 (treated)	17	0.183	1,400	272
1969 (treated)	16	0.136	740	. . .
1970 (treated)	15	0.126	940	196
1970 (untreated)	12	0.243	1,715	65

*Reproduced, with permission, from Levy DA. In: *Conference on the Biological Role of the IgE System.* Ishizaka K, Dayton DH Jr (editors). US Department of Health, Education, & Welfare, 1973.

†Includes only those patients for whom all 3 sets of data are available.

Note: SI X IgE Ab: $r_s = 0.94$ ($p = 0.02$). SI X IgG Ab: $r_s = 0.7$ ($p > 0.1$).
IgE Ab X IgG Ab: $r_s = 0.7$ ($p > 0.1$). r_s = symptom index.

sorbent and a standard patient's serum containing antibodies to that allergen, this allergen assay can be inhibited by similar unknown allergen solutions for standardization purposes. For example, a known system (such as ragweed antigen E) coupled to an immunosorbent and reacted with a known patient's IgE antiserum to ragweed antigen E gives a certain level of binding of radiolabeled anti-IgE. An aliquot of unknown allergen containing the same amount of qualitatively identical antigen E should theoretically inhibit this reaction 100% if added prior to the immunosorbent-bound antigen E. A lesser quantity of antigen E or a partially related antigen would cause less complete inhibition. Therefore, this test can be used to standardize unknown allergen preparations in both quantity and quality. Six WHO-standardized allergenic extracts have been accepted for standardization of such extracts manufactured anywhere in the world. Another dozen allergenic extracts are in process of being standardized for WHO. Methods of standardization are a combination of skin test end point titration, RAST inhibition, and isoelectric focusing of extracts to determine whether a minimal number of established important antigens are present.

IgE & Cell-Mediated Immunity

A reciprocal relationship between serum IgE antibody levels and cellular immunity has been demonstrated in rats immunized with dinitrophenyl-*Ascaris* extract. Thymus-derived lymphocytes appear to regulate the production of IgE, and immunization with *Ascaris* antigen and *Bordetella pertussis* vaccine stimulated T cells. A second immunization on day 5 with *Ascaris* protein conjugated with the dinitrophenyl hapten caused high titers of IgE antibody. Neonatally thymectomized or lethally irradiated rats could not make anti-dinitrophenyl IgE antibody. IgE antibody production was not restored with B cells alone but was restored with both T and B cells. Thus, T cells helped B cells turn on the IgE antibody mechanism.

Sublethal whole body irradiation, adult thymectomy and splenectomy, T cell immunosuppression with antithymocyte serum, and radiomimetic drugs (5-bromouridine deoxyriboside and dactinomycin) all enhanced IgE production, which lasted for weeks. It appeared that IgE production persisted in the absence of T cells. IgG and IgM antibodies occurred soon after immunization and did not stop IgE production. Next, thymus (or spleen) cells from hyperimmunized rats were injected into irradiated rats that were producing IgE antibodies. This caused a rapid fall (within 2 days) in IgE. Thus, IgE production by B cells appears to be under exquisite regulatory control by T cells and independent of the IgG, IgM, and IgA system.

IgE production in humans is under T cell regulatory control. A patient with thymic alymphoplasia, with a high serum IgE level (16 μg/mL) and allergic symptoms, had severe depletion of small lymphocytes in the thymic-dependent paracortical areas of lymph nodes, but lymphoid and plasma cells were normal. Patients with Wiskott-Aldrich syndrome have high serum IgE, eczema, and impairment of cellular immunity. Similarly, eczema also occurs in patients with ataxia-telangiectasia and impaired cellular immunity. Patients with Hodgkin's disease or sarcoidosis, with acquired impairment of cellular immunity, are being reported with high serum levels of IgE. On the other hand, patients with X-linked hypogammaglobulinemia (often with compensatory hyperactive cellular immunity) commonly have eczema.

Humoral and cellular immune responses were evaluated in 10 patients with active atopic dermatitis. IgE levels were elevated (mean, 6420 IU/mL; normal mean, 90 IU/mL), but complement receptor lymphocytes and surface immunoglobulin-positive B cells were normal. T cell function was diminished in that 6 out of 9 patients were unresponsive to concentrated *Candida* (1:10) and streptokinase-streptodornase (1:1) extracts as delayed skin tests. Spontaneous sheep erythrocyte T cell rosettes were below normal, and 2 of 10 patients had reduced PHA responsiveness. Eighteen patients with moderate to severe atopic dermatitis had a mean of only 13.7% active T cell rosetting lymphocytes compared to 29.6% in 30 normal adults. In both of these studies, all patients were being treated with topical corticosteroids, and these drugs could

have caused impaired cellular immunity. In summary, there appears to be an inverse relationship between IgE concentration and cellular immunity.

An interesting concept of "allergic breakthrough" has been proposed (Fig 15–6) in which IgE antibody production is minimal in normal nonallergic individuals and maintained at a low level by IgE "damping mechanisms" such as suppressor T cells or their soluble factors. The low level is maintained because the damping affects only the IgE system. In animals, experimental procedures such as low-dose irradiation, cyclophosphamide, and antilymphocyte serum treatments temporarily depress T cells, especially the normal regulatory suppressor T cells. Natural events, such as virus infections, appear to act in a similar manner. This depression permits escape of IgE helper T cells that stimulate IgE-forming B cells. Both T and B cells may then respond by differentiation and proliferation upon contact with a suitable antigen in the environment or diet. Therefore, depression or failure of the normal damping mechanism may initiate allergic sensitization.

Conversely, stimulation of IgE suppressor T cells may restore this damping mechanism and return IgE antibody levels to normal, ie, below the allergic threshold. In inbred mice, Freund's complete adjuvant has strongly stimulated IgE suppressor T cells to abrogate ongoing IgE antibody production. Other immunotherapy procedures may also stimulate this damping mechanism, as discussed later under immunotherapy.

REGULATION OF IgE ANTIBODY PRODUCTION

During the past decade, immunoregulation of IgE antibody formation has evoked great scientific interest, both for the basic elucidation of the mechanisms of immunoregulation in general and for the potential practical means of suppressing harmful antibodies. Furthermore, IgE immunoregulation appears to be, in part, controlled independently from IgM, IgG, and IgA responses.

Antigen in Freund's complete adjuvant (CFA) generally induces high levels of IgG and IgM antibodies. However, Katz observed that mice given antigen in CFA failed to make IgE antibodies; in fact, CFA appeared to suppress IgE antibody response. Serum from CFA-treated mice inhibited IgE production by normal mice. From such sera, Katz isolated a **suppressive factor of allergy (SFA)** that nonspecifically suppressed IgE antibody responses in mice to many antigens. Subsequently, an **"enhancing factor of allergy" (EFA)** was isolated; both SFA and EFA acted only upon the IgE system, with no regulatory effect on IgG, IgM, or IgA antibodies. A lectin affinity column with concanavalin A (Con A) failed to bind SFA but did bind EFA, which could then be eluted with excess mannose. Human SFA, found in supernates of pokeweed mitogen-stimulated cultured human lymphocytes, prevents or abrogates IgE production in mice (Table 15–3).

The cell sources of these **IgE regulatory factors** have been elucidated by several groups (Ishizaka, Kishimoto, Katz, and others). IgE added to splenic cultures enhanced the number of cells bearing Fc receptors for IgE ($Fc_\epsilon R$), especially in parasite-infected rats and mice or in newborn animals. In culture, lymphoid cells exposed to IgE produce soluble regulatory factors.

Kishimoto and coworkers primed Balb/c mice with DNP-hapten derivatives of mycobacteria (Myc) followed by immunization with alum-absorbed DNP-ovalbumin. Anti-DNP IgE response was suppressed in such animals primed with DNP-Myc. Suppressor T cells from such animals produced an IgE-specific suppressor factor (IgE-TsF); a hybridoma made from these cells spontaneously produced IgE-TsF. This had a molecular weight of 60,000, bound to lentil lectin, and had an antigenic determinant coded by the MHC complex (Table 15–3).

The Ishizaka group studied rats stimulated to form IgE following infection with the parasite *Nippostron-*

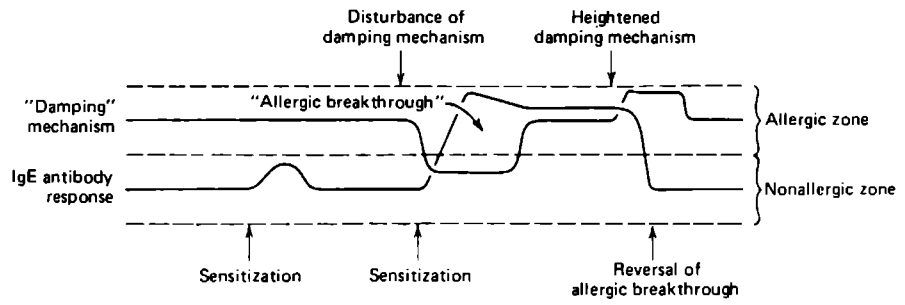

Figure 15–6. Possible pathogenesis of "allergic breakthrough." (Reproduced, with permission, from Katz DH et al: Regulation of IgE antibody production by serum molecules. 5. Evidence that coincidental sensitization and imbalance in the normal damping mechanism results in "allergic breakthrough." *J Immunol* 1979;122:2191.)

Table 15–3. Properties of IgE-specific regulatory factors.*

	SFA	EFA	IgE-TsF	IgE-Potentiating Factor	IgE-Suppressive Factor
Cell source	Lyt 1$^+$	T cells	Lyt 2$^+$	Lyt 1$^+$, Fc$_\epsilon$R$^+$†	Lyt 1$^+$†, Fc$_\gamma$R$^+$
Molecular weight	150,000	?	60,000 (150,000)	15,000	15,000
Affinity for IgE	–	–	+	+	–
Affinity for lentil lectin	–	+	+	+	–
MHC-restriction	–	–	+	–	–
Target cells	T cells	T cells	sIgE$^+$ cells‡	sIgE$^+$ B cells	sIgE$^+$ B cells Plasma cells

*Reproduced, with permission, from Ishizaka K: Regulation of IgE synthesis. Page 159 in: *Annual Review of Immunology 1984*. Annual Reviews, 1984. Copyright © 1984 by Annual Reviews Inc.

†w 3/25$^+$ T cells in the rat.

‡Suppress IgE synthesis by IgE-forming hybridoma.

gylus brasiliensis. Mesenteric lymph nodes (MLN) of these infected rats had T cells that made both **IgE-potentiating** and **IgE-suppressive factors,** each having a molecular weight of 15,000. These MLN T cells had W3/25 markers that correspond to mouse Lyt 1$^+$ cells. The IgE-potentiating factor had a mannose-rich oligosaccharide and an affinity for lentil lectin and Con A, while IgE-suppressive factor lacked such lectin affinity (Table 15–3).

When these MLN T cells with IgE were activated with 10 μg of Con A, they produced IgE-potentiating factor. However, if **tunicamycin,** which inhibits protein glycosylation, was added in a parallel culture, these cells produced IgE-suppressive factor. Thus, tunicamycin switched these T cells from forming IgE-potentiating factor to producing IgE-suppressive factor. In a similar manner, the glucocorticosteroid dexamethasone also switched such cells from IgE-potentiating to IgE-suppressive factor production. Glucocorticoids induced biosynthesis of **lipomodulin,** a phospholipase inhibitory protein. MLN T cells activated with 10 μg of Con A made IgE-suppressive factor when incubated with IgE and lipomodulin, instead of the expected IgE-potentiating factor. With only 1 μg of Con A, activated MLN T cells incubated with IgE made IgE-suppressive factor; however, in the presence of melitin or a monoclonal antilipomodulin, which activate phospholipase, such cells switched to make IgE-potentiating factor. Therefore, the same activated MLN T cells in the presence of IgE made either IgE-suppressive factor or IgE-potentiating factor, depending upon the pharmacologic environment.

Although there are similar effects among these various IgE immunoregulatory factors, they have somewhat different biochemical properties and order of action, as indicated in Table 15–3. Katz showed that neither SFA nor EFA has affinity for IgE, but both appear to regulate the IgE response through the formation of IgE-suppressive and IgE-potentiating factors.

Substances that induced IgE-binding factors from macrophages of adjuvant-treated animals appear to be interferonlike. Mouse β interferon induced normal mouse spleen cells to form IgE-binding factors. Normal mouse spleen cells co-cultured with measles virus-infected HeLa cells, which have type I interferon, induced normal rat lymphocytes to form IgE-binding factors; this induction was prevented by anti-type I interferon antibodies. Antigen-induced T cells stimulated by antigen may induce IgE-binding factors via gamma interferon.

Ishizaka and coworkers have described a **glycosylation-inhibition factor (GIF)** which appears to be a 15,000-MW fragment of phosphorylated lipomodulin from CFA-KLH-primed spleen cells, including macrophages and T cells, which induced spleen cells to act on unprimed T cells to form IgE-suppressive factor (Fig 15–7). A rat-mouse T cell hybridoma that formed IgE-suppressive factor upon incubation with IgE spontaneously released GIF. Glucocorticoids induce macrophages to form a 15,000-MW phospholipase inhibitory protein "macrocortin," which binds to antilipomodulin. Thus, it appears that GIF is a fragment of phosphorylated lipomodulin or "macrocortin" that selectively forms IgE-suppressive factor.

Spleen cells of rats primed with KLH in alum or normal rat spleen cells incubated with "pertussigen" (lymphocytosis-promoting factor [LPF]) from *Bordetella pertussis* cause the release of **glycosylation-enhancing factor (GEF).** Incubation of normal rat MNL cells with IgE and GEF resulted in selective formation of a glycosylated IgE-binding factor that enhanced IgE response. GEF appears to be a kallikreinlike enzyme that cleaves a substrate on or in T lymphocytes to form a kininlike material that enhances glycosylation of IgE-binding factors through activation of phospholipase.

The nature and biologic activities of IgE-binding factors formed by the same T cells are determined by the balance between GEF and GIF, which induces the formation of IgE-potentiating or IgE-suppressive factors. Katz's EFA and SFA may be similar or identical to GEF and GIF, respectively.

Ishizaka suggests the schema in Fig 15–7. Stimulation of receptor-bearing T cells (Fc$_\epsilon$R or Fc$_\gamma$R) by either IgE or interferonlike inducers results in formation of both IgE-potentiating factor and IgE-suppressive factor. The presence of kallikreinlike enzyme, GEF, enhances the assembly of N-linked oligosaccharide to

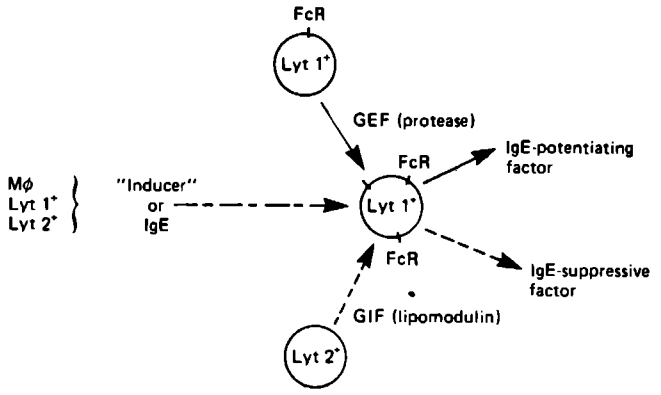

Figure 15–7. Schematic models for the selective formation of IgE-potentiating factor or IgE-suppressive factor. FcR$^+$ Lyt 1$^+$ T cells form IgE-potentiating factor in the presence of GEF, but the same cells form IgE-suppressive factor in the presence of GIF. (Reproduced, with permission, from Ishizaka K: Regulation of IgE synthesis. Page 159 in: *Annual Review of Immunology 1984*. Vol 2. Annual Reviews, 1984. Copyright ©1984 by Annual Reviews Inc.)

form IgE-potentiating factor. In the presence of a fragment of phosphorylated lipomodulin (GIF), lack of glycosylation favors formation of IgE-suppressive factor. Therefore, a balance between GEF and GIF near FcR$^+$ T cells ($Fc_\epsilon R$ or $Fc_\gamma R$) will direct which IgE-binding factor is formed, and these factors will either enhance or suppress the IgE response.

Although the detailed dissection of these IgE immunoregulatory mechanisms has been done in rodents, similar activated *human* T cells form IgE-binding factors when incubated with IgE. Patients with hyper-IgE syndrome or atopic dermatitis have peripheral blood T cells that produce IgE-potentiating factor which has an affinity for human IgE. Identification of similar IgE immunoregulatory factors in humans and their potential production by human T cell hybridomas, especially of IgE-suppressive factor, promise a means for fine-tuning human IgE responsiveness and control of IgE-mediated human atopic diseases.

TARGET CELLS OF IgE-MEDIATED ALLERGIC REACTIONS

Mast Cells

Basophilic cells in connective and subcutaneous tissues were first recognized by Ehrlich, who associated a "feeding" or "mast" function with them. Others described them as "emergency kits" or "sentinal cells" of mediator-effector responses placed at mucous and cutaneous surfaces and around venules to recruit critical factors in homeostasis of the microenvironment (Fig 15–8). Human skin and gastrointestinal tract are particularly rich in mast cells (7000/μg and 20,000/μg, respectively). The primary function of mast cells appears to be storage of granules containing potent inflammatory and repair materials that are released upon injury to the organism. They contain strong pharmacologically active materials such as heparin, histamine, serotonin, kinin protease, and SRS-A; tissue degradation and repair materials such as chymase and hyaluronic acid; and factors affecting other cells, such as eosinophil and neutrophil chemotactic factors of anaphylaxis (ECF-A, NCF-A) and platelet-aggregating factor. It has been generally accepted that mast cells in all tissues are the same.

Heterogeneity among mast cells is becoming apparent in both rats and humans. Typical **connective tissue mast cells** are located near blood vessels. They are large (> 20 μm) with many granules that have a high histamine content (> 15 pg/cell). Histamine is bound to a highly sulfonated proteoglycan and heparin. In addition to degranulation induced by immunologic reactions, connective tissue mast cells are readily degranulated by nonspecific agents such as compound 48/80, polymyxin, and opiates. Cromolyn sodium, theophylline, and ketotifen block connective tissue mast cell degranulation, which makes these drugs clinically useful.

In rat gastrointestinal mucosa, Enerbach described the **mucosal mast cell,** often called a "globule leukocyte" by others. Mucosal mast cells are lysed in formalin, which causes them to be missed in usual histologic sections. They are preserved with basic lead acetate fixation and stain well with 1% alcian blue and 0.1% eosin. Mucosal mast cells are smaller (< 10 μm) and have fewer granules with low histamine content (< 2 pg/cell). Histamine is bound to a low sulfonated proteoglycan without heparin but with an unidentified monoamine. They are found in all layers of the gastrointestinal tract but are abundant in the mucosa. Parasite infection, eg, with *N brasiliensis*, in the rat causes a lymphoblast influx that brings about a marked proliferation of mucosal mast cells. They may transform from or respond to a soluble factor from helper T cells.

Both connective tissue and mucosal mast cells have surface receptors that bind IgE antibodies, and upon antigen exposure they degranulate to release media-

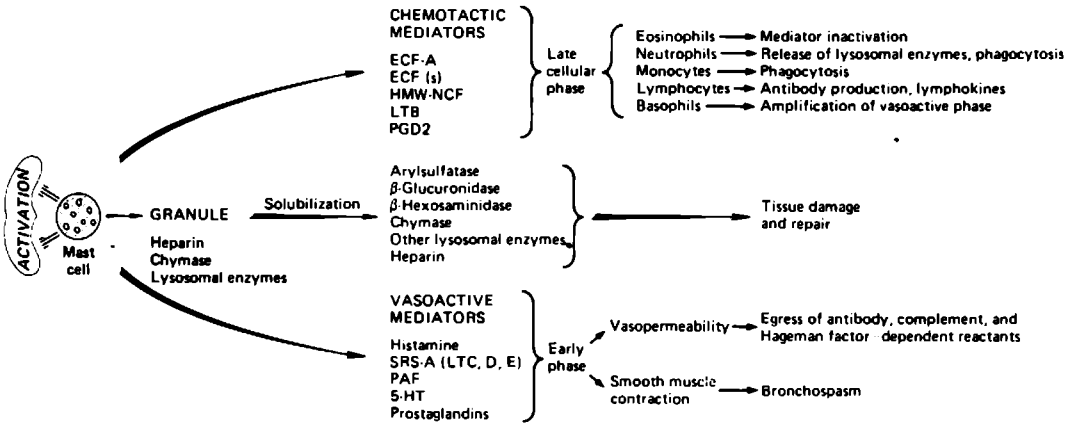

Figure 15–8. Schematic view of relationship between mast cell activation and inflammatory events. (Reproduced, with permission, from Wasserman SI, Soter NA: Page 192 in: *Advances in Allergology and Applied Immunology*. Pergamon Press, 1980.)

tors. In contrast to connective tissue mast cells, mucosal mast cells resist degranulation by compound 48/80, polymyxin, and opiates. In rats, cromolyn sodium and theophylline do not block antigen-induced degranulation of mucosal mast cells, although doxantrazole and flavonoids such as quercetin appear to block mucosal mast cell degranulation. In humans, cromolyn may inhibit both kinds of mast cells, which may explain its effectiveness in human lung, where mucosal mast cells appear predominant.

Human and rat gastrointestinal tracts have varying proportions of connective tissue and mucosal mast cells from mucosal surface to adventitia and along the length of the tract. Mouth, tongue, esophagus, and proximal stomach are rich in connective tissue mast cells and have few or no mucosal mast cells. The distal stomach, duodenum, jejunum, and especially ileum and colon have abundant mucosal mast cells in which the lamina propria and submucosa are particularly rich. This distribution of cells might explain the relative ineffectiveness in the prevention of food allergy of cromolyn sodium, which blocks connective tissue mast cells in the relatively nonabsorptive esophagus and stomach. Perhaps doxantrazole or quercetin, either of which blocks mucosal mast cells in the absorptive small intestine, would be more useful.

Basophils

Basophilic cells make up 0.5–2% of circulating white cells. They were once thought to be identical to mast cells, from which they are indistinguishable by light microscopy, but electron microscopy reveals that the structure of granules is different in the 2 types of cells. Basophils contain histamine, leukotrienes, and NCF-A but no PGD_2. Their histamine release is not inhibited by cromolyn sodium.

Other Target Cells

Blood platelets contain serotonin and possibly other allergic inflammatory mediators. In some species, like the rabbit, platelets also contain heparin and histamine. Histamine released from rabbit platelets is involved in the deposition of IgG immune complexes in the renal tissue of rabbits with acute glomerulonephritis. Such immune complex deposition can be prevented by prior treatment of the rabbit with antihistamine drugs. Furthermore, neutrophils and macrophages participating in the allergic inflammatory response are major sources of SRS-A leukotrienes, prostaglandins, and kinins, and perhaps other secondary mediators.

MEDIATORS OF ALLERGIC REACTIONS

Physiologic Role of Allergic Mediators

The primary role of the mediators of allergic reactions appears to be defense against injury, first by causing inflammation and then by stimulating tissue repair. In the presence of large concentrations of antihistamines, surgical wound healing is considerably impaired. Heparin temporarily suspends blood clotting, which permits inflammatory cells to enter the area of tissue injury. Histamine causes leakage by blood vessel endothelial cells and permits additional inflammatory cells and serum proteins, such as antibody, to enter the area of damaged tissue. There appears to be a role for histamine and perhaps other mediators in normal growth—especially fetal growth. The highest concentrations of histamine are present during the fetal period. In pregnant rats given chronic high doses of antihistamines, fetal growth and maturation were impaired. Histamine appears to have a role in the control of microcirculation at the capillary level, where there appears to be a homeostatic balance between capillary constriction caused by epinephrine and capillary dilatation caused by histamine.

Although diseases such as acute glomerulo-

nephritis are associated with deposition of antigen-IgG-complement complexes in the kidney or lung, these may be harmful overreactions of a normal defense mechanism. In intestinal parasitic nematode infestations in the rat, IgE on mast cells reacts with parasitic antigens. This causes the release of histamine and serotonin, which results in leakage of serum proteins from intestinal blood vessels. Among such proteins are IgG and IgM antibodies against the parasite, and these antibodies are apparently involved in the normal clearing of parasites from the intestine. Therefore, this is a useful defense mechanism. In animals, the acquisition of IgE antibodies to parasites results in permanent protection against subsequent infection by that parasite. This is known as the "self-cure phenomenon."

Facilitation by mediators of other cells entering the field of reaction may augment inflammation or may function in negative feedback control of the inflammation. The release of ECF-A causes the influx of eosinophils into the area of allergic inflammation. Electron micrographs have shown eosinophil phagocytosis of ferritin-antibody complexes.

Pharmacologic Role of Mediators

A. Histamine: Histamine is a bioactive amine (MW 111) that causes smooth muscle contractions of human bronchioles and small blood vessels, increased permeability of capillaries, and increased secretion by nasal and bronchial mucous glands. Histamine is stored in granules of mast cells and basophils and is released upon dissolution of the granules. Its maximal reaction occurs in 1–2 minutes, and its duration of action is about 10 minutes. Therefore, in humans it can be responsible for the symptoms of hay fever, urticaria, angioedema, and the bronchospasm of acute anaphylaxis.

B. Serotonin: Serotonin, or 5-hydroxytryptamine (MW 176), occurs in murine mast cells and human platelets. It has a pharmacologic role in anaphylaxis in mice, rats, and rabbits but apparently not in humans. It is antagonized by lysergic acid. Serotonin is preformed, is held in mast cell granules, and, similar to histamine, is released as a result of an antigen-antibody reaction.

C. ECF-A: ECF-As (eosinophil chemotactic factors of anaphylaxis), 2 acidic tetrapeptides with molecular weights of about 500, are also released as a result of an antigen-antibody reaction. They are preformed, like histamine and serotonin, and upon release they cause an influx of eosinophils into the area of allergic inflammation. The eosinophils dispose of antigen-antibody complexes and may exert feedback controls on mediators.

D. HMW-NCF: HMW-NCF, high-molecular-weight (about 750,000) neutrophil chemotactic factors, are formed after bronchial challenge with antigen and in physical urticarias, eg, cold and solar. They attract neutrophils specifically but not eosinophils or mononuclear cells and appear to be important in late-phase reactions.

E. Kinin-Generating Proteases: Three proteases from human basophils and lung fragments cleave and activate Hageman factor to generate kinins from plasma kininogen and by prekallikrein activation. These proteases may link IgE-mediated reactions with Hageman factor-dependent kinin-generating coagulation and fibrinolytic pathways. **Bradykinin** is a 9-amino-acid peptide split by the enzyme kallikrein from a serum α_2-macroglobulin precursor. In humans, it causes slow, sustained contraction of smooth muscles, including those of the bronchi and vessels; increased vascular permeability; increased secretion of mucous glands, including those of the bronchi; and stimulation of pain fibers. Therefore, bradykinin could be responsible for symptoms of hay fever, angioedema (associated with painful swelling), and asthma.

F. Platelet-Activating Factors (PAF): PAF (MW 523 and 551) is the common name for a substance identified structurally and synthesized by Pinckard as acetyl glycerol ether phosphorylcholine (AGEPC) and by Benveniste as PAF-acether. It is released by IgE antibody-antigen reactions, by non-IgE reactions from rabbit and human basophils, and from human alveolar macrophages. In guinea pigs, PAF-acether is the most potent bronchoconstrictor described so far. In humans, it is postulated that intrinsic asthma might occur by PAF-acether released by non-IgE reactions from alveolar macrophages that aggregate platelets; these release granules containing potent bronchoconstrictors. Within 15 seconds after AGEPC infusion in baboons, intravascular platelet aggregation and release of platelet factor 4 (PL4) and thromboxane B_2 (TXB_2) occur with marked increase in pulmonary artery pressure and prolonged systemic hypotension resembling anaphylactic shock in humans. AGEPC is 100–1000 times more active on a molar basis than histamine in causing wheal and flare in human skin.

G. Arachidonic Acid Metabolites: Cell membrane phospholipids are phosphorylated during IgE-mediated reactions that activate phospholipases A_2 and C to form arachidonic acid. This in turn is oxidized in either of 2 pathways—5-lipoxygenase or cyclooxygenase—that appear interrelated. Slow-reacting substances are generated in inflammatory reactions from several cell sources.

H. Slow-Reacting Substance of Anaphylaxis (SRS-A): SRS-A results from antigen-antibody interactions. It has been identified as a mixture of extremely potent spasmogenic and vasodilatory lipoxygenase metabolites of arachidonic acid; these are the **leukotrienes** LTC_4, LTD_4, and LTE_4 (Fig 15–12F). They appear to be generated in 2 phases after an IgE antibody-antigen reaction. Pure human mast cells generate 5–10 units of SRS-A per microgram of cellular histamine released, while human lung fragments generate 40–100 units of SRS-A per microgram of cellular histamine released. This suggests a combined action of mast cells with other activated cells, probably pulmonary interstitial mononuclear cells, to form SRS-A; this appears to be a mixture of the 3 leuko-

trienes. Leukotrienes are chiefly involved in the continued bronchospasm of asthma.

Leukotrienes have 100 times (LTC_4) to 1000 times (LTD_4) the potency of histamine as bronchial smooth muscle spasmogens on isolated muscle strips. In vivo, leukotrienes, especially LTD_4, cause a marked prolonged fall in dynamic compliance of peripheral airways in guinea pigs, whereas the histamine effect chiefly influences central airway resistance. LTD_4 causes hypotension in the guinea pig, while both LTD_4 and LTE_4 are about 100 times more potent than histamine in causing vascular permeability in guinea pig skin.

From membrane arachidonic acid in human neutrophils, 5-lipoxygenase generates 5-hydroxyeicosatetraenoic acid (5-HETE), which modulates cell motility and possibly glucose transport; and LTB_4 which is a potent chemotactic agent comparable to C5a.

Both oxidative pathways of arachidonic acid are inhibited by an analog, 5,8,11,14-eicosatraynoic acid (ETYA), while benoxaprofen and piriprost block only the 5-lipoxygenase pathway. The cyclooxygenase pathway is inhibited by nonsteroidal anti-inflammatory agents, eg, aspirin, indomethacin, and ibuprofen.

I. Prostaglandins and Thromboxanes: These result from cyclooxygenase metabolism of arachidonic acid. Human lung mast cells preferentially form PGD_2, a potent vasodilator. From neutrophils and macrophages, this pathway generates $PGF_{2\alpha}$, a potent bronchoconstrictor, and PGE_1 and PGE_2, potent broncho- and vasodilators that regulate the tissue microenvironment. PGI_2 causes disaggregation of platelets, while thromboxanes (TXA_2 and TXB_2) aggregate platelets and thus are potent regulators of blood coagulation and homeostasis.

There appear to be considerable physiologic reinforcing interactions between 5-lipoxygenase and cyclooxygenase pathway reagents. In human skin, PGD_2 causes a transient edema (wheal and flare) which, if given with a subclinical dose of the chemotactant LTB_4, causes prolonged neutrophilic induration. Inhibition of one of the 2 pathways may make more arachidonic acid substrate available for metabolism by the other pathway. It was proposed that cyclooxygenase inhibition by aspirin made more arachidonic acid available to form SRS-A leukotrienes to cause aspirin-induced asthma. However, benoxaprofen, a 5-lipoxygenase inhibitor, did not prevent aspirin-induced bronchospasm. Therefore, the interactions of arachidonic acid metabolites require more study to further elucidate mechanisms of action.

Histamine Release From Sensitized Leukocytes (Basophils)

A practical in vitro miniature allergic reaction is used extensively as an investigative and diagnostic test for allergy. Leukocytes from a heparinized blood sample drawn from an allergic individual are incubated with the allergen for 15 minutes. The leukocyte sample is centrifuged, and the histamine content of the supernate is measured spectrofluorometrically (or by radiolabeled histamine or bioassay). With increasing amounts of antigen, there is increased histamine release. The histamine is expressed as a percentage of the total amount of histamine present in an aliquot of leukocytes. Blood basophils are the only source of histamine in the blood, and although a buffy coat preparation is used, one is actually measuring basophil histamine release. The degree of sensitivity in a patient is directly proportionate to the amount of histamine released by antigen. The degree of sensitivity in different patients may be compared by the relative amounts of antigen required to release 50% of the histamine from their white cells. A remarkable correlation between the degree of symptoms and the amount of antigen necessary for 50% release from leukocytes has been noted (Fig 15–9). This test has been useful in following the course of patients during hyposensitization therapy (Fig 15–10). At the start of therapy, the patient in Fig 15–10 required 5 μg of antigen to release 50% of his histamine, whereas after 2½ years of therapy he required 5150 μg of antigen to release the same percentage of histamine. This is a 1000-fold decrease in his cell sensitivity and correlated positively with his clinical improvement.

Eosinophils

Eosinophils are intimately associated with allergic reactions, and blood or local tissue eosinophilia is of-

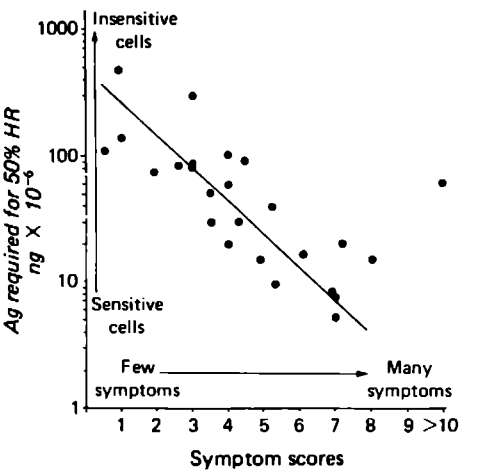

Figure 15–9. Correlation between average seasonal symptom scores of hay fever patients and measurements of cellular sensitivity to antigen E (nanograms of antigen required for 50% histamine release [HR] from a standard suspension of isolated washed leukocytes). (Reproduced, with permission, from Lichtenstein LM, Norman PS: Human allergic reactions. [Editorial.] *Am J Med* 1969;46:169. Also in: Norman PS: Present status of hyposensitization treatment. Page 46 in: Proceedings of the Sixth Congress of the International Association of Allergology. Excerpta Medica Foundation, 1968.)

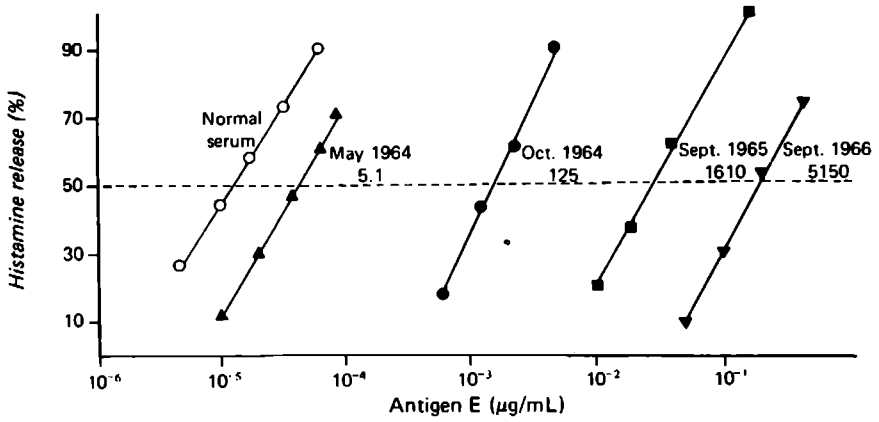

Figure 15–10. Dose-response curves of cells suspended in normal serum or in the patients's own serum obtained before immunotherapy (May 1964) and after approximately 1, 2, and 3 years of immunotherapy. The numbers to the right of the curves in allergic serum indicate the ratio of the antigen concentration required for 50% histamine release in the allergic sera as compared to the normal serum (open symbols). (Reproduced, with permission, from Lichtenstein LM, Norman PS: Human allergic reactions [Editorial.] *Am J Med* 1969; 46:170.)

ten a useful clinical test for allergy or parasitic infestation. Eosinophils normally comprise about 1–3% of the circulating leukocytes; in allergic patients, 10–20% eosinophilia may occur.

Blood and tissue eosinophilia is a common feature of parasitic infection and allergic reactions. Helminth infections in animals and humans evoke both IgE and IgG antibodies, which activate eosinophils and neutrophils for antibody-dependent cell cytotoxicity to the parasites, especially larvae. IgE antiparasite antibodies on mast cells react with parasite antigens to release vasoactive amines and chemotactants for eosinophils (ECF-A) and neutrophils (NCF-A). Activated eosinophils act as executioners for helminth larvae by depositing their highly toxic granular contents on the larval integument. High pH granule proteins, major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO) cause direct damage to parasites. Depletion of eosinophils with antieosinophil serum causes loss of immunity to parasites and enhanced numbers of larvae in murine *Schistosoma* and *Trichinella* infections; therefore, eosinophils appear to be cardinal in defense against helminths.

The potent destructive materials in eosinophil granules prompted Gleich and others to investigate their role in allergic tissue damage. Mouse monoclonal and rabbit antibodies to animal and human MBP, ECP, and EPO have been made. By radioimmunoassays, asthmatics had elevated levels of MPB in sera and sputa. Using immunofluorescent anti-MBP serum, both abundant intraeosinophil and extracellular deposits of MBP were found in epithelium, sputum casts, and submucosa of airways on autopsies of 11 asthmatics but not in controls. Human and rat pneumocytes in culture are detached by EPO and lysed by high concentrations of MBP. Both human and horse eosinophils produce significant amounts of leukotrienes LTC_4 and LTD_4. Human eosinophils produced about 5 times the amount of LTC_4 as did neutrophils, so that eosinophils may be an important source of bronchoconstrictive leukotrienes in asthmatics.

Eosinophils in Late-Phase Asthmatic & Allergic Reactions

The classic view of the pathogenesis of allergy is that allergen induces formation of IgE antibody which sensitizes mast cells and basophils. Upon reexposure to allergen, IgE bridging causes degranulation of the cells with release of allergic mediators. Thus, therapy has been directed at neutralizing allergic mediators with antihistamines and other antimediators (diethylcarbamazine for SRS-A) and β-adrenergic and xanthine bronchodilators. However, this simple schema does not explain allergic symptoms without apparent immediate exposure to antigen, nasal priming where small or inconsequential exposures to antigen cause symptoms following a major reaction to an unrelated antigen, and the effectiveness of topical corticosteroids in treatment.

Late-phase reactions have been recognized in IgE-sensitized skin; following an immediate reaction lasting 30–60 minutes, there is often a 4- to 8-hour late reaction of erythema, induration, and burning pain. In the IgE-sensitized nose and bronchi, the immediate sneezing, itching, and wheezing after antigen challenge lasts 0.5–2 hours, followed in 4–8 hours by obstructed airways, wheezing, and poor response to bronchodilators. Such late-phase reactions could account for the recrudescence of symptoms in the absence of new antigen exposure, such as in nocturnal asthma and rhinitis, and prolonged or chronic symptoms lasting 24–48 hours after allergen exposure.

Bronchial hyperreactivity and eosinophils were observed in human asthmatics who manifest both early and late responses (dual responders) but not in asthmatics who manifest only early responses (single re-

sponders). On bronchoalveolar lavage, eosinophils did not change in either group after methacholine inhalation. However, after antigen inhalation in dual responders, there was a significant rise in eosinophils at 24 hours. The serum MBP level rose significantly in all and persisted for 24 hours in dual responders. There was a significant inverse correlation between increased eosinophils in bronchoalveolar lavage fluid to the dose of methacholine that caused a 20% drop in forced expiratory volume at 1 second. Similarly, in mite-allergic asthmatics, deMonchy found large numbers of eosinophils in bronchoalveolar lavage 6–7 hours after mite allergen inhalation and an increased ECP:albumin ratio in bronchoalveolar lavage fluid, suggesting eosinophil degranulation and secretion of products.

Eosinophils and their secretory products cause damage in other tissues, especially the skin. In chronic urticaria, extracellular MBP deposition was found in connective tissues. In atopic dermatitis, bright eosinophils with MBP and extensive extracellular MBP fibrillar staining in the upper dermis were found. Pathogenesis of atopic dermatitis has been suggested of IgE-induced cutaneous late-phase reactions with dermal eosinophil degranulation and MBP deposition. In hypereosinophilic syndrome, death occurs from heart failure resulting from Löffler's eosinophilic endomyocarditis with diffuse extracellular MBP in subendomyocardium. In human lung, skin, and heart, eosinophils and their secreted granular products have caused tissue damage from allergic and possibly other mechanisms.

A possible role of eosinophils in the pathogenesis of late or chronic asthma has major implications for clinical management. Corticosteroids depress blood and sputum eosinophilia, so that part of their effectiveness in chronic asthma or in damping the later inflammatory phase of acute status asthmaticus may be due to their antieosinophil effect. Cromolyn sodium inhalation before mite antigen inhalation in asthmatics with late-phase reaction depressed numbers of eosinophils found in bronchoalveolar lavage fluid. Both corticosteroids and cromolyn depress bronchial hyperreactivity to methacholine and histamine inhalation. Therefore, in management of the inflammatory phase of chronic asthma, corticosteroids and cromolyn may be the drugs of choice rather than bronchodilator drugs, as is current common practice.

In late asthmatic responses, early and intermediate neutrophil and eosinophil inflammation is superseded by mononuclear cells, which are the principal cells recovered in bronchoalveolar lavage fluid after 24 or 48 hours following antigen inhalation. Activation of monocytes and neutrophils after allergen-induced asthma was studied by measuring changes in C3b receptors on such cells. In atopic asthmatics, antigen inhalation caused a significant drop in FEV_1, accompanied by a sustained 65% rise in neutrophil chemotactic activity (HMW-NCF-A). Concomitantly, there was a slower, highly significant rise in C3b rosettes on both monocytes (> 70%) and neutrophils (> 46%) at 30 and 60 minutes postchallenge. These results suggested that mast cell mediators activated inflammatory cells after antigen challenge. Activation of granulocytes and monocyte-macrophages can cause tissue damage and increased bronchial lability or can reactivate mast cells for further mediator release. In late-phase reactions, mononuclear cell infiltration may be important in sustaining granulocyte-induced tissue damage and bronchial hyperreactivity.

Autonomic Nervous Controls as "Mediators" of Allergic Reactions

The catecholamines (epinephrine, norepinephrine, dopamine) are agonists of the sympathetic nervous system; acetylcholine is the parasympathetic agonist. These substances modulate the severity of the allergic reaction by regulating the amount of mediator release from target cells and the degree of responsiveness of shock organ cells. Catecholamines contain a catechol nucleus (a benzene ring with 2 adjacent hydroxy groups) and an amine group. Epinephrine acts primarily as a hormone, and norepinephrine is a neurotransmitter at both peripheral and central levels. Both compounds occur naturally in mammals. Another synthetic catecholamine, isoproterenol, has important pharmacologic effects on the allergic reaction.

The autonomic nervous system is divided into sympathetic (adrenergic) and parasympathetic (cholinergic) systems, and these exert generally opposing actions on the various organs of the body. These 2 systems maintain homeostasis in bronchial smooth muscle cells; cholinergic stimulation through acetylcholine causes smooth muscle constriction, whereas adrenergic stimulation through epinephrine causes relaxation. Thus, there is constant alternating responsiveness of bronchial smooth muscles to cholinergic and adrenergic stimuli, maintaining smooth muscle tone or homeostasis.

CELL RECEPTORS & ALLERGIC REACTIONS

Biochemical Cellular Events in Release of Allergic Mediators From Mast Cells & Basophils

A. IgE Receptor: The receptors for IgE in basophils and mast cells have been characterized in rat basophil leukemia (RBL) cells, mouse mastocytoma cells, and human basophils. Schematically, Fig 15–12A shows the 2-chain IgE receptor (MW 80,000) composed of α (MW 50,000) and β (MW 30,000) chains, each of which has 2 domains. In the larger α chains, both α_1 and α_2 domains appear on the basophil surface. The smaller α_1 (MW 21,000) is rich in carbohydrate; the larger α_2 (MW 24,000) has little carbohydrate but apparently binds to the IgE molecules. The nonglycosylated intramembranous noncovalently bound β chain has β_1 (MW 20,000) and β_2 domains; the latter appears exposed on the inner cytoplasmic side of the plasma membrane. Phosphoryla-

tion of both the α and β chains has been reported, but whether this actually does occur and if so how are currently under study.

B. Triggering Signals Induced by IgE Receptor Bridging: A rabbit antiserum against IgE receptors of RBL cells (anti-RBL) was produced that replicated all the effects of antigen-IgE antibody or IgE-anti-IgE reactions in basophils. Fragments $F(ab)'_2$ of IgE antibody with antigen, or of rabbit anti-RBL with receptor, or of rabbit anti-IgE with IgE all caused histamine release, whereas their Fab' monomers failed to do so (Fig 15–11). Therefore, bridging of two IgE receptors was needed for histamine release; IgE antibody with antigen or anti-IgE with IgE acted merely as a suprasurface projection of the IgE receptors.

Both second messenger systems—cAMP and intracellular calcium (Ca^{2+})—are simultaneously activated by several pathways in basophils and mast cells. (Most of the research in this area has been done on rat peritoneal mast cells and cultured rat basophil leukemia cells.) Two or more IgE receptors are brought together on the surface of the plasma membrane by antigen and IgE antibodies, by anti-IgE serum, or by IgE receptor antibodies. In the cAMP system (Fig 15–12A), the apposed IgE receptors (R_S) stimulate GTP-dependent stimulatory G-protein (G_S), which in turn stimulates inactive adenylate cyclase to dephosphorylate ATP to cAMP. Four molecules of cAMP attach to 2 regulatory dimers (R) of inactive protein kinase A, causing a steric change that releases 2 catalytic subunits (C) of active protein kinase A. This active protein kinase A can release some bound Ca^{2+} in the endoplasmic reticulum into the cytosol to act as second messenger. By phosphorylation, active protein kinase A also directly converts inactive cell protein into active cell protein (protein-P), which in moderate Ca^{2+} concentrations increases—and in high Ca^{2+} inhibits—mediator release. In similar fashion, stimulation of the β-adrenergic receptor with epinephrine analogs elicits the production of G_S, which causes adenylate cyclase to convert ATP to cAMP, and the same events occur as above. Generally, cAMP in larger amounts inhibits mediator release.

Several natural or pharmacologic agents inhibit this cAMP system. *Bordetella pertussis* toxin inhibits GTP dephosphorylation. From other cell systems, a stimulus in high concentration (such as putative high anti-IgE antibody) stimulates an inhibitor receptor (R_i), which stimulates an inhibitory G-protein (G_i) that inhibits adenylate cyclase; GTP is required. This reaction is turned off by GTPase, forming GDP. However, cholera toxin blocks GTPase, thus prolonging adenylate cyclase activity. G_S and G_i stimulate and inhibit adenylate cyclase, respectively, but their action is blocked by *B pertussis* toxin, also altering the action of adenylate cyclase. Finally, breakdown of cAMP into AMP by phosphodiesterase is inhibited by xanthines (theophylline, caffeine), which preserve cAMP; these are the classic drugs used to treat asthma.

The second pathway for raising cytosolic Ca^{2+} second messenger (Fig 15–12B) involves plasma membrane inner leaflet that forms phosphatidylinositol 4, 5-bisphosphate (PIP_2) by successive phosphorylations from ATP of plasma membrane inositol (I) to phosphatidylinositol (PI) to phosphatidylinositol 4-phosphate (PIP) to PIP_2. The approximated IgE receptors (described above) activate the GTP-dependent G-protein (G_S) that with the G-dependent phosphodi-

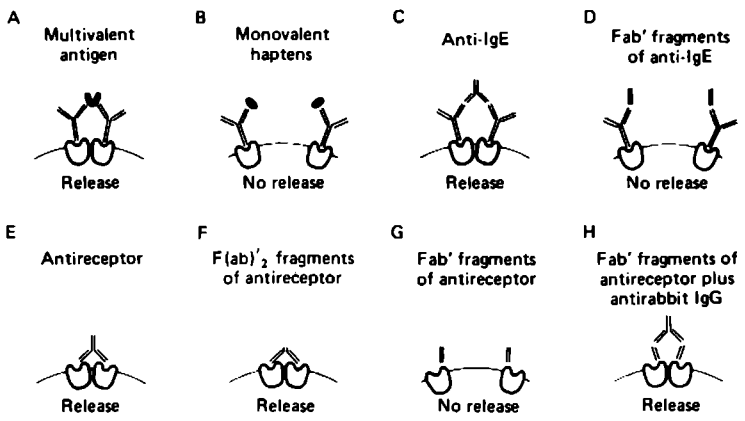

Figure 15–11. Schematic summary of studies on the roles of IgE and IgE receptor in mediator release from mast cells and basophils. *A.* Multivalent or divalent antigens initiate release. *B.* Monovalent haptens do not initiate release. *C.* Anti-IgE initiates mediator release *D.* Monovalent Fab' fragments of anti-IgE do not initiate release. *E.* Antibodies to IgE receptor initiate release. *F.* Divalent $F(ab)'_2$ fragments of antireceptor antibody also cause release. *G.* Monovalent Fab' fragments of the rabbit antireceptor antibody do not initiate release unless *(H)* they become linked by antirabbit IgG antibody. (Reproduced, with permission, from Kulczycki A Jr: Role of immunoglobulin-E and immunoglobulin-E receptors in bronchial asthma. *J Allergy Clin Immunol* 1981; **68**:5.)

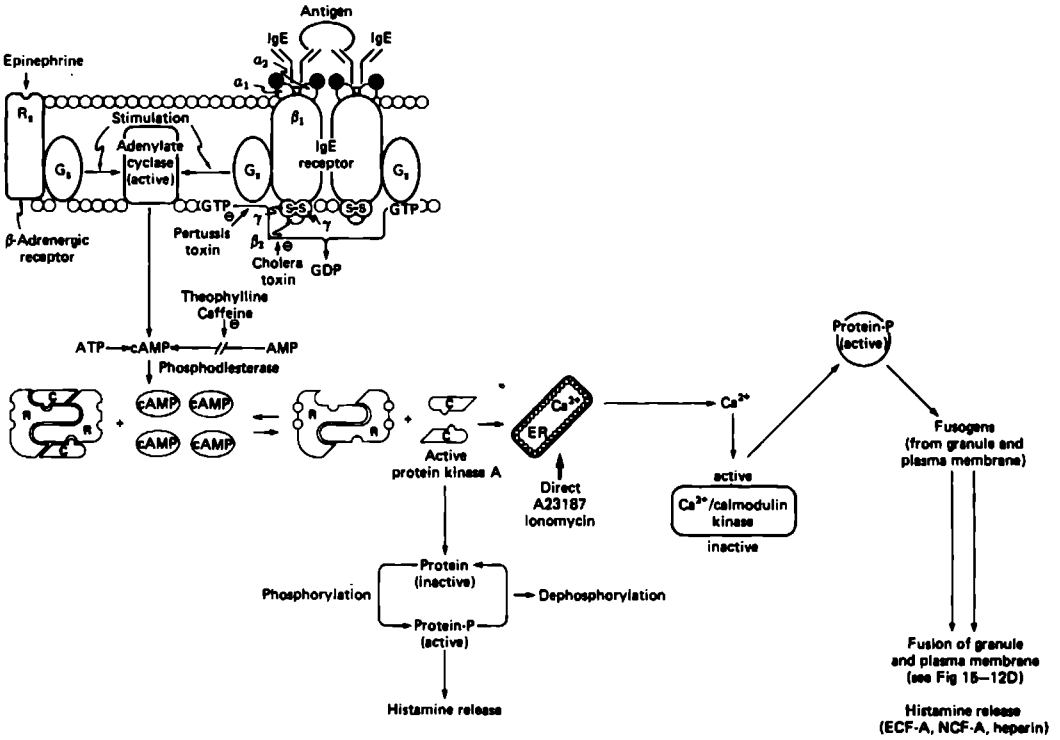

Figure 15–12A. IgE-Fc receptor-linked adenylate cyclase and β-adrenergic receptor activation of adenylate cyclase for activation of cAMP-dependent protein A-kinase for initiation or inhibition of mediator release from mast cells and basophils. GDP = guanosine diphosphate; GTP = guanosine triphosphate; C = catalytic subunit; R = regulatory subunit; ⊖ = inhibition. (Reproduced, with permission, from Metzger H et al: Structure of the high-affinity mast cell receptor for IgE. *Fed Proc* 1982; 41:8.)

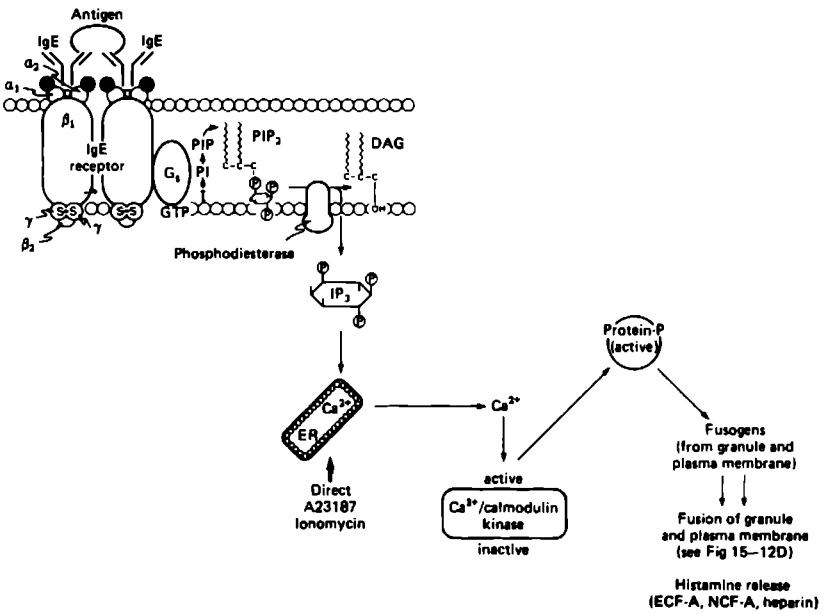

Figure 15–12B. Activation of cytosolic Ca^{2+} second messenger by formation of PIP_2 from inositol and cleavage of IP_3, which releases Ca^{2+} from endoplasmic reticulum to activate Ca^{2+}/calmodulin-sensitive phosphorylase kinase for fusogen protein-P activation and mediator release. A23187 = calcium ionophore; DAG = diacylglycerol; ER = endoplasmic reticulum; GTP = guanosine triphosphate; I = inositol; IP_3 = inositol triphosphate; PI = phosphatidylinositol; PIP = phosphatidylinositol 4-phosphate; PIP_2 = phosphatidylinositol 4,5-bisphosphate. (Adapted from Berridge MJ: The molecular basis of communication within the cell. *Sci Am* [Oct] 1985; 253:142.)

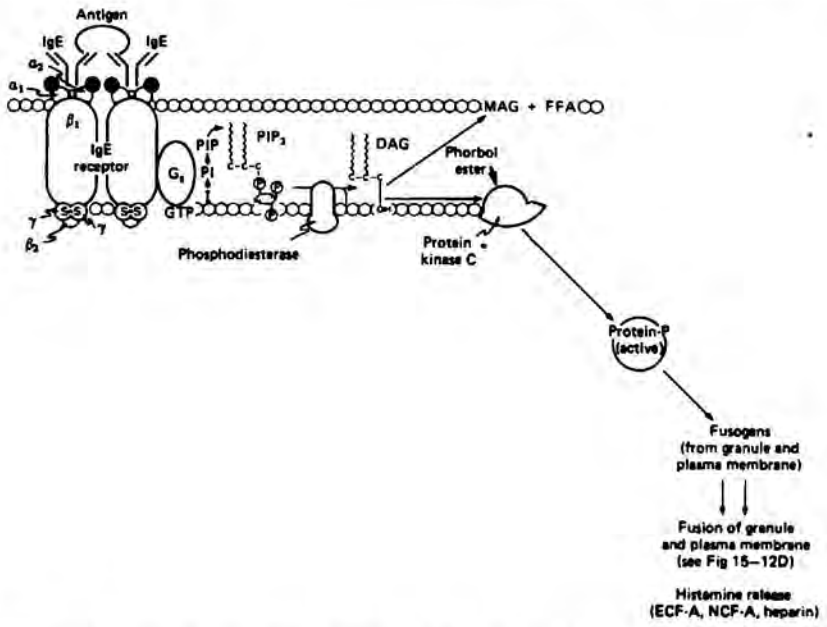

Figure 15–12C. Formation of diacylglycerol from cleavage of PIP_2 for activation of protein kinase C (phosphokinase C) and fusogen formation. DAG = diacylglycerol; FFA = free fatty acids; I = inositol; MAG = monoacylglycerol; PI = phosphatidylinositol; PIP = phosphatidylinositol 4-phosphate; PIP_2 = phosphatidylinositol 4,5-bisphosphate. (Adapted from Berridge MJ: The molecular basis of communication within the cell. *Sci Am* [Oct] 1985; 253:142.)

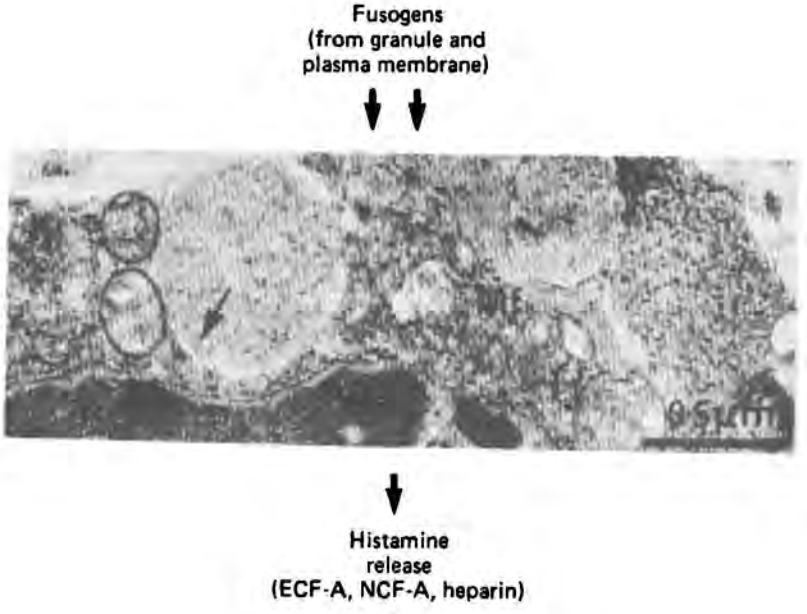

Figure 15–12D. Ultrastructural view of mast cell granule membrane fusion with plasma membrane and extrusion of granule contents. (Reproduced, with permission, from Trotter CM, Orr TS: A fine-structure study of some cellular components in allergic reactions. 1. Degranulation of human mast cells in allergic asthma and perennial rhinitis. *Clin Allergy* 1973; 3:411.)

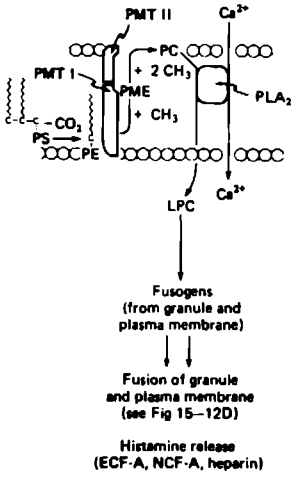

Figure 15–12E. IgE-receptor stimulation activates and methylates plasma membrane phospholipids for calcium channel and lysophosphocholine fusogen formation. LPC = lysophosphocholine; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PLA_2 = phospholipase A_2; PME = phosphatidyl-N-monomethyl ethanolamine; PMT = phospholipid methyltransferase. (Reproduced, with permission, from Hirata F, Axelrod J: Phospholipid methylation and biological signal transmission. *Science* 1980; 209:1087.)

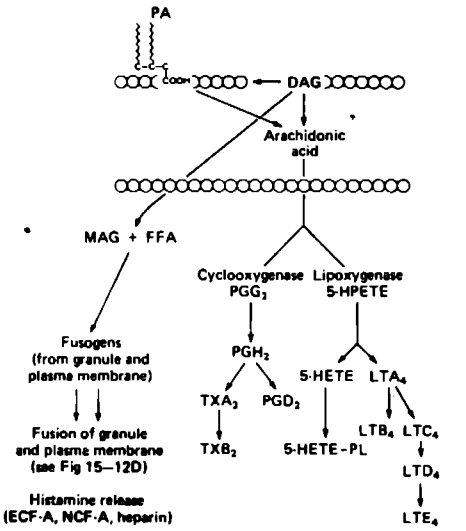

Figure 15–12F. Formation of arachidonic acid from membrane triglycerides by diacylglycerol and subsequent formation of cyclooxygenase and lipoxygenase mediators. DAG = diacylcerol; FFA = free fatty acids; HETE = hydroxyeicosatetraenoate; HPETE = hydroperoxyeicosatetraenoate; LT = leukotriene; MAG = monoacylglycerol; PA = phosphatidic acid; PG = prostaglandin; PL = phospholipid; TX = thromboxane. (Reproduced, with permission, from Lewis RA, Austen KF: Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. *Nature* 1981; 293:103.)

esterase (phospholipase C) splits PIP_2 into membrane-bound diacylglycerol (DAG) and inositol triphosphate (IP_3), which is soluble in the cytoplasm. This cytosolic IP_3 acts to free Ca^{2+} bound in the endoplasmic reticulum—an action similar to that of active protein kinase A from the cAMP pathway. Ca^{2+} can also be released from the endoplasmic reticulum directly with ionophore A23187 or ionomycin. This cytosolic increased Ca^{2+} (from either pathway) binds to and activates a Ca^{2+}/calmodulin-sensitive phosphorylase kinase, which in turn phosphorylates a cellular cytosolic protein (protein-P), which acts as a membrane fusogen. It is not yet clear whether this is the same protein-P that is directly activated by protein kinase A in the cAMP system; they may be members of a family of Ca^{2+}-binding proteins—calelectrins—such as synexin and endonexin.

Activated protein-P and other fusogens are also formed from the second DAG wing of the PIP_2 cleavage by the phospholipase C pathway (Fig 15–12C). DAG alone can react with plasma membrane protein kinase C in a minimal fashion. However, in conjoint action of DAG with inner leaflet membrane phosphatidylserine (PS), a marked activation of protein kinase C causes greatly increased phosphorylation and activation of cytosolic protein-P fusogen formation. Phorbol esters can stimulate protein kinase C directly. Furthermore, DAG is cleaved by diacylglycerol lipase into monoacylglycerol (MAG) and free fatty acids (FFA), both of which are fusogens.

Fusogens (activated protein-P, MAG, FFA) (Fig 15–12D) cause fusion of the activated mast cell or basophil granule membranes into large granules that come to the mast cell or basophil surface and fuse with the plasma membrane and cause extrusion of the granule contents into the extracellular fluid. These granule contents include preformed mediators such as histamine, serotonin (in murine rodents), heparin, and chondroitin sulfate and the chemotactants ECF-A and high- and low-molecular-weight NCF-A.

Stimulation of the IgE receptor and G_S activates inner leaftlet membrane PS to decarboxylate to phosphatidylethanolamine (PE) (Fig 15–12E). PE is methylated once by methyltransferase I to monomethyl PE, and also—nearer the external membrane surface—with 2 additional methyl groups by methyltransferase II to phosphatidylcholine (PC). Membrane phospholipase A_2 (PLA_2) then converts PC to lysophosphatidylcholine (LPC), which is another fusogen. PLA_2 also creates an open calcium channel in the plasma membrane through which extracellular Ca^{2+} enters the cytosol to greatly increase the concentration of intracytoplasmic Ca^{2+}; this further abets granule and plasma membrane fusion and exocytosis of preformed mediators.

Finally, DAG directly or in concert with PLA_2 act-

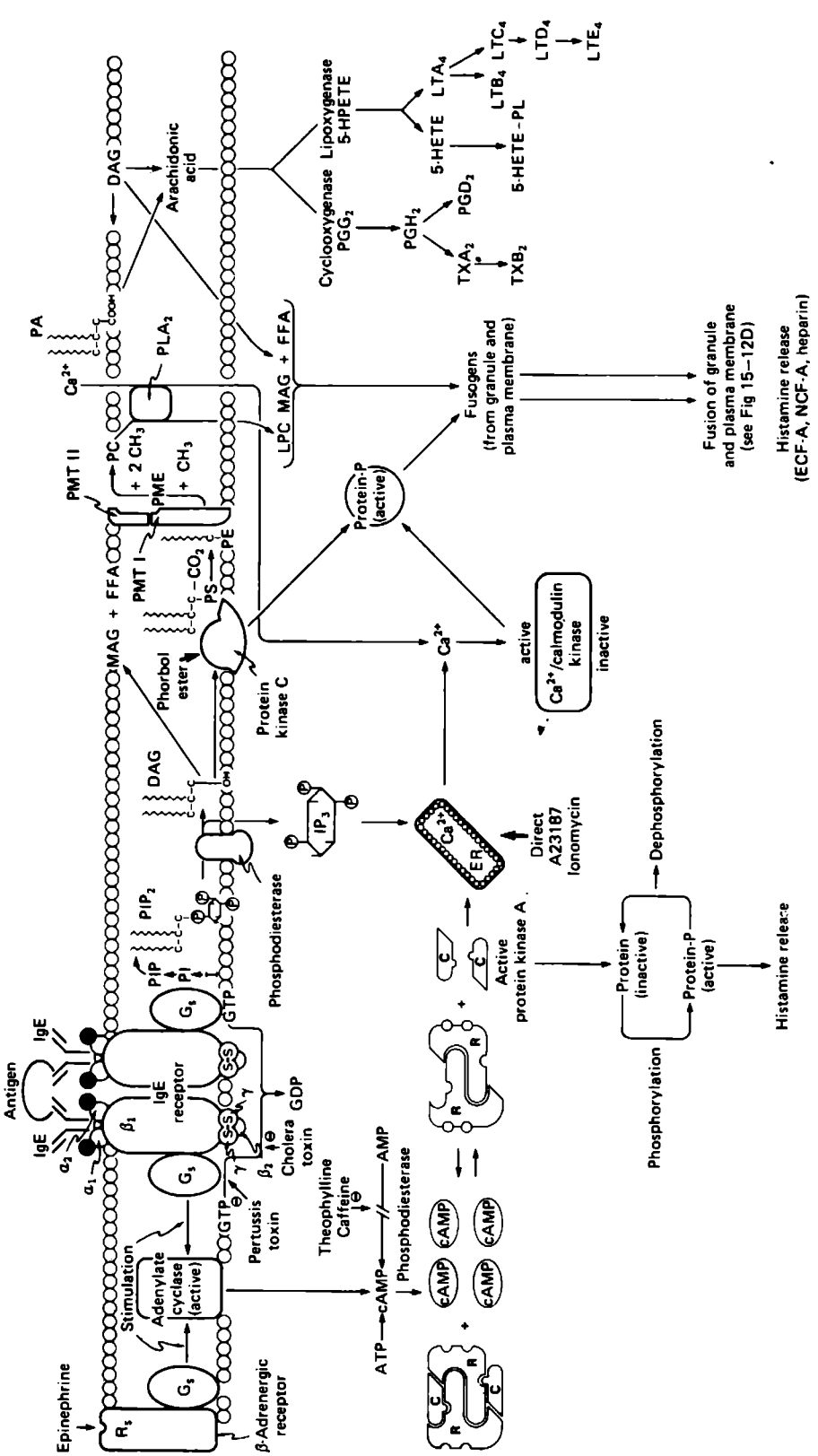

Figure 15–12(A–F). Composite view of interrelationships of intracellular pathways leading to mediator release from mast cells and basophils after IgE-receptor stimulation.

ing on phosphatidic acid (PA) liberates arachidonic acid from membrane triglycerides (Fig 15–12F). Arachidonic acid is acted upon by 2 enzyme systems—cyclooxygenases and lipoxygenases—to produce newly formed mediators. From cyclooxygenase action, intermediary prostaglandins (PGG_2 and PGH_2) and thromboxanes (TXA_2) are formed, leading to release of mediators PGD_2 and TXB_2. From lipoxygenase activity, intermediate 5-HPETE (hydroperoxyeicosatetraenoate) leads to formation of 5-HETE (hydroxyeicosatetraenoate) phospholipid, which promotes fluidity, and leukotrienes LTA_4 to LTB_4 (chemotactants) and LTC_4, LTD_4, and LTE_4 (slow-reacting smooth muscle constrictors). Fig 15–12 (A–F) presents a composite view of the interrelationships among these pathways in mast cells and basophils after allergen-IgE antibody interaction, leading to the formation and release of various mediators of allergic inflammation.

When anti-RBL serum was added to RBL cells in the presence of preincubated methyl ^{3}H-methionine, methyl ^{3}H-lysophosphatidylcholine appeared within 15 seconds, indicating the speed of the phospholipid methylation upon bridging of IgE receptors. In these stimulated cells, within 2 minutes $^{45}Ca^{2+}$ uptake and within 3 minutes histamine release were maximal (Fig 15–13). S-Isobutyl-3-deazoadenosine (3-deazo-SIBA) inhibits S-adenosyl-L-methionine-mediated methylation. In purified rat mast cells preincubated with 1–100 μmol of 3-deazo-SIBA and exposed to anti-receptor (RBL) serum, all 3 reactions—methyl ^{3}H-lysophosphatidylcholine formation, $^{45}Ca^{2+}$ uptake, and histamine release—were blocked, which suggests that all 3 reactions depend on phospholipid methylation.

Concomitantly with membranous phospholipid methylation, membrane adenylate cyclase is stimulated by the bridging of two IgE-receptors aided by increased fluidity of the plasma membrane. In the presence of Ca^{2+}, activated catalytic protein converts cytosol ATP to cAMP. This peak rise in cAMP occurs within 15 seconds—concomitantly with phospholipid methylation, described above—and results in feedback suppression of further phospholipid methylation and inhibition of further Ca^{2+} influx and histamine release (Fig 15–14).

Clinical Relevance in Asthma of IgE Receptor Concentration on Mast Cells

In order to explain clinical differences in allergic respiratory diseases, it has been proposed that pollen- or dander-sensitive asthmatics have many IgE receptors on mast cells that react with many high-affinity IgE antibodies directed at multivalent pollen antigens, eg, ragweed antigen E (Fig 15–15). Such reactions would occur even with minute doses of pollens, because of a profusion of ragweed IgE antibodies; these would be bimolecular reactions occurring at a rate dependent upon concentration of antigen and its receptor-bound specific IgE antibodies.

With house dust mite or mold allergens, mast cells might contain only a few scattered IgE receptor-bound antibody molecules in which a high concentration of antigen would be necessary to form sufficient bridges to appose two IgE receptors and trigger the reaction. This might proceed as a trimolecular reaction which would be proportionate to:

[Antigen] X [Receptor-bound antigen-specific IgE]^2.

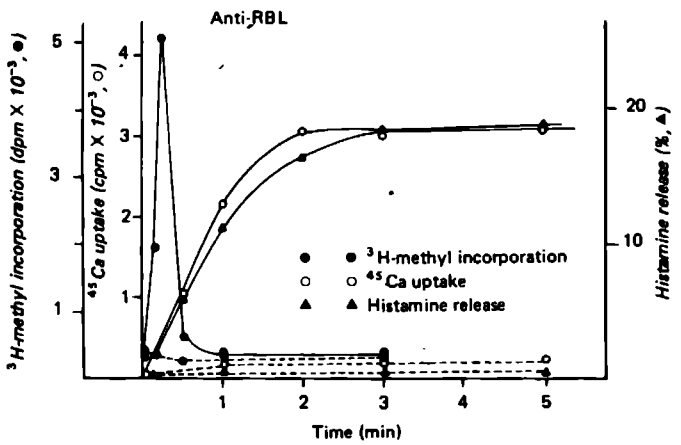

Figure 15–13. Phospholipid methylation, Ca^{2+} influx, and histamine release in rat mast cells. Mast cells were first incubated with [^{3}H]methionine or $^{45}Ca^{2+}$. Incorporation of [^{3}H]methyl groups into phospholipids, $^{45}Ca^{2+}$ influx, and histamine release were measured after various treatments. Cells were treated with either divalent F(ab)'$_2$ (—) or monovalent Fab' (- - -) fragments of antibodies to RBL cells. (Reproduced, with permission, from Ishizaka T: Biochemical analysis of triggering signals induced by bridging of IgE receptors. *Fed Proc* 1982; 41:17.)

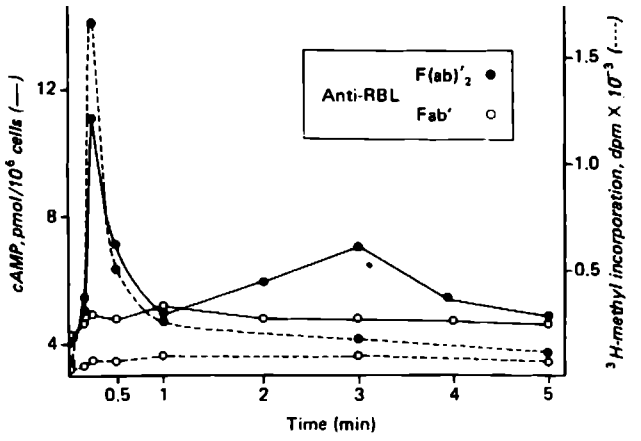

Figure 15–14. Kinetics of changes in cAMP (—) and phospholipid methylation (- - -) induced by either F(ab)'$_2$ fragments (●) or Fab' fragments (○) of anti-RBL. (Reproduced, with permission, from Ishizaka T: Biochemical analysis of triggering signals induced by bridging of IgE receptors. *Fed Proc* 1982; 41:17.)

Therefore, in patients with chronic or intrinsic asthma, a specific antigen exposure might be unrecognized because rapid reactions would occur only in times of extremely high antigen exposure, eg, changing a vacuum cleaner bag or bronchial inhalation challenge with house dust. IgE levels usually wane in the elderly, and therefore it is thought that "idiopathic" or "intrinsic" asthma in this age group may be related to increased house dust or mold.

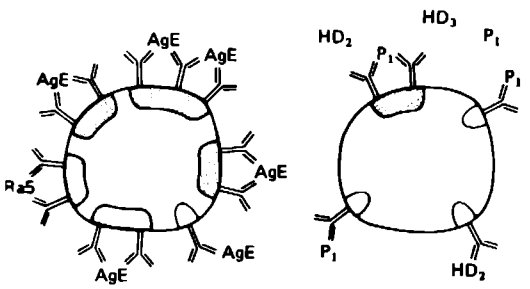

Figure 15–15. Hypothetical schematic model illustrating that clinical differences in chronicity of allergic diseases may be related in part to differences in numbers of receptor molecules bridged by antigen. Unoccupied receptors and receptors occupied by other IgE molecules are not shown. Bridged (or apposed) receptors that initiate mediator release are shaded; unapposed receptors are not. *Left:* Mast cell of ragweed-allergic individual has many receptors occupied by IgE antibodies directed at multivalent ragweed antigens, predominantly antigen E (AgE). *Right:* Mast cell of house dust-allergic individual. In this case fewer antigen-specific IgE antibodies are present that are directed at antigen P_1 and other theoretic house dust (HD) antigens. (Reproduced, with permission, from Kulczycki A Jr: Role of immunoglobulin-E and immunoglobulin-E receptors in bronchial asthma. *J Allergy Clin Immunol* 1981; 68:5.)

Receptors for Autonomic Agonists

Target cells have receptors, presumably on their surfaces, for the autonomic agonists acetylcholine and epinephrine. Adrenergic receptors apparently have a receptive substance with a steric configuration complementary to the amine group in the catecholamine. This interaction initiates a chain of biochemical reactions which culminate in the ultimate reaction of that cell. Although receptors have not as yet been biochemically defined, they are usually described in terms of actions induced by agonists and by blockage of such actions by antagonistic agents. On this basis, Ahlquist described adrenergic α and β receptors that often had antagonistic or synergistic effects. α-Adrenergic receptors respond primarily to norepinephrine, with epinephrine or isoproterenol causing a 10- or 100-fold reduced response, respectively. On the other hand, β-adrenergic receptors respond primarily to isoproterenol, with epinephrine and norepinephrine similarly causing a 10- or 100-fold reduced response. Thus, epinephrine is a natural agonist for both α and β receptors, with its ultimate effect probably modulated by other factors. α-Adrenergic receptors are blocked by ergot alkaloids, haloalkylamines (eg, phenoxybenzamine, dibenamine), benzodioxans, and imidazolines. β-Adrenergic receptors are blocked by propranolol, dichloroisoproterenol, and butoxamine.

β-Adrenergic receptors have been further classified, depending upon their action on certain tissues, into β_1 and β_2 receptors. β_1-Adrenergic stimulation causes an increase in heart rate (chronotropic effect) as well as an increased force of cardiac contraction (inotropic effect). Increased mobilization of free fatty acids from fat cells also occurs, producing a rise in blood lipids. β_2-Adrenergic receptors cause relaxation of smooth muscles, especially in the bronchus, uterus, and bladder. They also cause inhibition of peripheral glucose uptake and increased muscle glycogenolysis, both of which cause a rise in blood glucose. The ago-

nist for both β_1 and β_2 receptors is isoproterenol; both are blocked by propranolol. There are, however, several newly synthesized agonists for β_2 adrenergic receptors, eg, metaproterenol (Alupent, Metaprel), albuterol (Salbutamol), and terbutaline (Bricanyl). These have been developed for treatment of asthma to act primarily as bronchodilators and to avoid the cardiac stimulatory effects of β_1 receptors. The β_2 receptors are blocked specifically by butoxamine, while pronethalol has primarily a β_1 blocking action.

Whether α-adrenergic receptors exist in the human bronchus is a question under current study, and there is some disagreement. In animals such as the guinea pig, α-adrenergic stimulation by norepinephrine or propranolol blocking of β-adrenergic receptors results in bronchoconstriction. Such an effect has not been conclusively demonstrated in humans. In summary, the allergic shock organ tissues, smooth muscle, and vascular endothelium have responses modulated by the autonomic nervous system.

The β-adrenergic receptor is a cell membrane enzyme coupled to a G-protein that stimulates adenylate cyclase. This enzyme is changed from a relatively inactive to an active form by isoproterenol or epinephrine. Activated adenylate cyclase catalyzes the formation of cyclic nucleotide (as shown in Fig 15–16) by acting upon ATP in the cell cytoplasm to form cAMP. cAMP in the presence of dibasic cations (Ca^{2+} and Mg^{2+}) modulates the activity of cell enzymes and permeability barriers. Furthermore, cAMP is converted to an inactive 5′-AMP by phosphodiesterase.

Intracellular concentration of cAMP rises in the target cell upon external stimulation with prostaglandins PGE_1 and PGE_2 and also increases with histamine itself (Fig 15–17). There appears, therefore, to be a catalytic subunit of adenylate cyclase in the membrane which responds to one of 3 or more independent receptor subunits. Such receptor subunits can be blocked independently by drugs—eg, β-adrenergic receptor is blocked by propranolol; the histamine re-

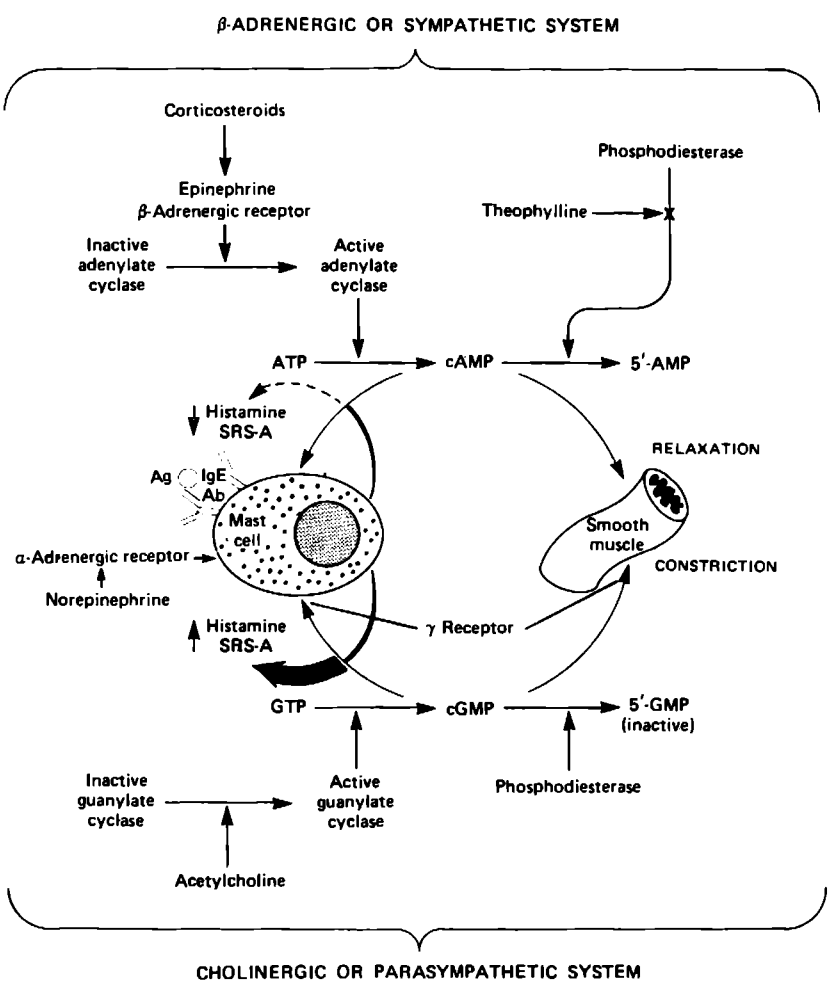

Figure 15–16. The balance theory of sympathetic and parasympathetic regulation.

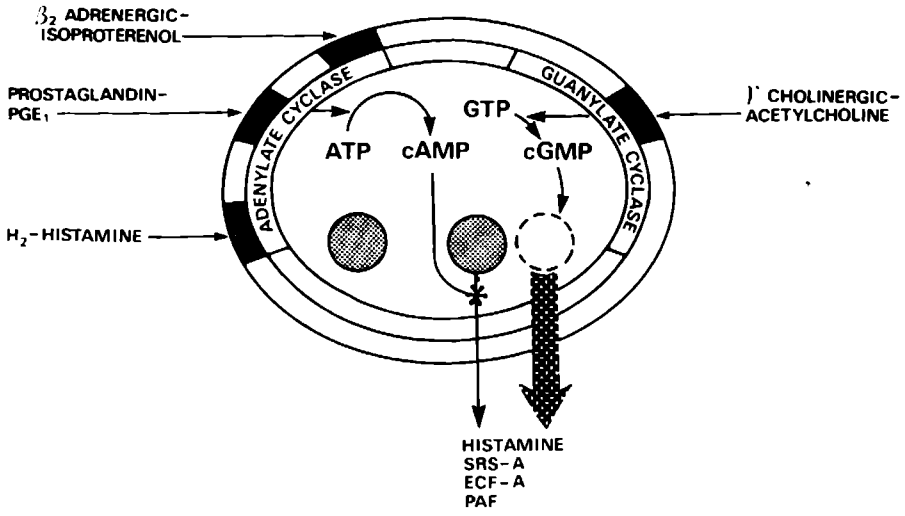

Figure 15–17. Schematic representation of a cell showing the relationships between intracellular cAMP and various independent receptor subunits.

ceptor is of the H_2 type which is blocked by burimamide; and the prostaglandin receptor may possibly be blocked by indomethacin or by aspirin. Basophils, in which the β-adrenergic receptor is blocked by propranolol, will still respond to prostaglandins PGE_1 or PGE_2 with a rise in intracellular cAMP—and also to exogenous histamine in a similar manner—whereas burimamide-treated basophils fail to respond to histamine but will respond with a rise in intracellular cAMP to epinephrine or PGE_1 stimulation. It has been suggested that the release of histamine from mast cells by antigen and antibody will exert a negative feedback control upon other basophils to inhibit further release of histamine. The concept of 3 independent receptors on target cells causing a rise in intracellular cAMP is of possible major therapeutic interest in status asthmaticus, wherein the β-adrenergic receptor appears to be unresponsive to epinephrine but is still apparently responsive to prostaglandin PGE_1.

Lymphocytes which form antibodies or which participate in cellular immune responses by undergoing mitogenesis and enlargement in response to antigen or plant lectins are under similar autonomic and biochemical regulatory controls. A rise in intracellular cAMP induced by epinephrine or prostaglandin PGE_1 in such lymphocytes inhibits mitogenesis and aborts further immune responsiveness. Therefore, hormonal influences upon antibody formation and immune response are under intensive current study.

The α-adrenergic receptor is less well understood. Several workers have suggested that ATPase may function as the α receptor. It may compete for ATP with adenylate cyclase in the direct formation of inactive 5′-AMP without the intermediary cAMP formation step. Norepinephrine may stimulate ATPase to utilize ATP directly to form inactive 5′-AMP; such a diversion of precursor ATP would cause a lowering of the intracellular cAMP, and this in turn would result in an increased mediator release from such a stimulated basophil.

Phosphodiesterase converts cAMP to inactive 5′-AMP. Methylxanthine drugs such as theophylline, theobromine, and caffeine inhibit phosphodiesterase and its destruction of cAMP; therefore, a high cAMP level is maintained. Leukocytes from ragweed-allergic individuals exposed to ragweed antigen E had a dose-dependent release of histamine. This was inhibited by increasing doses of epinephrine and, independently, by increasing doses of theophylline. In fact, the effect of these 2 drugs was synergistic in that ineffective inhibitory doses of either epinephrine alone or theophylline alone, when given together, resulted in important inhibition of histamine release. This synergistic effect of catecholamines and theophylline in the treatment of bronchospasm in asthma has been known for many years and was the rationale for using combinations of these drugs in single tablets for bronchodilator therapy of asthma. Currently, they are given individually in dosages calculated according to body weight.

The cholinergic or γ receptor in target cells appears to be guanylate cyclase, which occurs either in the membrane or in the cell cytoplasm. Inactive guanylate cyclase is converted by acetylcholine to active guanylate cyclase, and this converts guanosine triphosphate (GTP) into a cyclic nucleotide called cyclic 3′,5′-guanosine monophosphate (cGMP). In mast cells, the intracellular rise in cGMP is associated with an increase in mediator release. This release can be blocked by atropine. Similarly, in bronchial smooth muscle, a rise in intracellular cGMP is associated with smooth muscle contraction which is blocked by atropine. cGMP is destroyed by phosphodiesterase by conversion to an inactive form, 5′-GMP, or guanyl

monophosphate. Although both adenyl phosphodiesterase and guanyl phosphodiesterase are destroyed by the methylxanthines, it has been demonstrated that adenyl phosphodiesterase is about 10 times more susceptible to methylxanthine than is guanyl phosphodiesterase. This may explain the preferential action of methylxanthines on the cAMP system over that on the cGMP system and therefore the effectiveness of methylxanthines in the treatment of asthma.

In summary, the biochemical modulation of mediator release from mast cells and basophils has been demonstrated using preparation of IgE-sensitized cells prepared from minced human lung or human basophils and animal mast cells and basophils.

THE BALANCE THEORY OF REGULATION CONTROLS

A balance has been proposed between cAMP and cGMP to maintain homeostatic controls of cell activation. This is illustrated in Fig 15–16. In an asthmatic individual, cholinergic stimulation through acetylcholine acting upon guanylate cyclase causes a rise in cGMP. In the mast cell, mediator release is augmented and the bronchial smooth muscle constricts. This mechanism is blocked by atropine. Guanyl phosphodiesterase inactivates the elevated cGMP, converting it to 5′-GMP and turning off the response. Asthmatic symptoms may be further augmented by norepinephrine, which stimulates the α-adrenergic receptor to decrease cAMP and therefore augments the release of mediators from mast cells and possibly increases smooth muscle bronchoconstriction. This mechanism can be blocked by an α-adrenergic blocking agent such as phentolamine. Opposing these mechanisms is the cAMP system, in which epinephrine, isoproterenol, PGE_1 or PGE_2, and even histamine activate adenylate cyclase, which converts ATP to cAMP. This elevated cAMP level inhibits the release of mediators from the sensitized mast cells and results in bronchial smooth muscle relaxation. Methylxanthines such as theophylline further augment the cAMP level by inhibiting adenyl phosphodiesterase to prevent the destruction of cAMP. One action of corticosteroid hormones appears to be the improvement of epinephrine function on the β-adrenergic receptor to augment cAMP levels. This schema of a balance between cGMP and cAMP in asthma suggests that there are points in the mechanism which are likely to respond to pharmacologic control and could thus be of significance in treatment.

THE β-ADRENERGIC BLOCKADE THEORY OF ATOPY

It has been proposed that the basic problem in asthmatic individuals is a partial β-adrenergic blockade. This may be an inherited or acquired (ie, infectious) lesion of the receptor-transducer-adenylate cyclase complex, resulting in its defective function. Such a blockage would interfere with the homeostatic control of bronchi and target cells (mast cells and basophils). Either β-adrenergic blockage or cholinergic overreactivity could produce an autonomic imbalance and could account for increased airway irritability, for hypersensitivity to allergic mediators, for reduced sensitivity to catecholamines, and possibly for increased IgE production in certain allergic individuals.

Central Nervous System Reflexes in Patients With Asthma

Central neural control of bronchi is superimposed upon the biochemical sympathetic and parasympathetic agents acting upon target cells and smooth muscle cells. There are superficial irritant receptors in bronchi of animals and of humans. These receptors are stimulated by inhalation of irritating chemicals, such as sulfur dioxide and histamine, and by physical irritants such as cold air. Afferent fibers via the vagus nerve reach the brain stem, where they synapse with efferent vagal fibers returning to the bronchial smooth muscle. Stimulation of irritant receptors leads to a vagal reflex arc via the brain stem that results in reflex bronchial smooth muscle contraction and bronchospasm. In asthmatic individuals, these irritant receptors are hyperreactive, which is apparently the basis for the methacholine test. Asthmatic patients usually respond with bronchconstriction to inhalations of 0.25 mg methacholine, whereas the nonallergic individual will rarely react to even 25 mg of this compound. A positive methacholine inhalation test is used as a differential diagnostic test for asthma.

In a dog model of asthma, which responds with bronchoconstriction after exposure to inhaled antigen, deposition of antigen into one bronchus via a tube caused bronchospasm in both lungs. This indicates that a reflex arc controlling bronchospasm exists. As a result of an antigen-antibody reaction on the surface of a mast cell in the bronchus, histamine is released which acts upon irritant receptors to initiate the vagal reflex, resulting in bronchospasm. This is an alternative to the direct effect of histamine on bronchial smooth muscles. Parenteral or locally administered atropine blocks the bronchospasm following an antigen inhalation challenge in such asthmatic dogs and in similar allergic humans. Cooling blocks vagus impulses. In an asthmatic dog with the vagi surgically exposed and cooled with ice water, antigen-induced bronchospasm could be stopped within seconds by cooling the vagus. When the vagus was warmed, bronchospasm returned promptly, only to be terminated on recooling the vagus. The relative degree of the importance of the vagal reflex bronchospasm versus direct histamine-induced bronchospasm in asthma is currently under study by several groups.

APPROACHES TO THE TREATMENT OF ALLERGY BASED UPON THE MECHANISM OF THE REACTION

The first point of attack on the allergic mechanism is identification of the specific allergen by a careful history and skin tests, with subsequent avoidance of the allergen (Fig 15–18). This approach is frequently successful in the treatment of allergy due to animal danders, house dust, molds, or foods. The next point of attack is the antigen-antibody reaction on the surface of the mast cell. Immunotherapy or hyposensitizing injection of the allergen stimulates the formation of an IgG blocking antibody, which remains in the circulation or tissues. Upon exposure to antigen, these antibodies react with the antigen, forming a complex which is then removed by the reticuloendothelial system (Fig 15–19). If the antigen cannot reach the IgE on the target cells, the allergic reaction does not take place. In some patients, the clinical improvement correlates well with the degree of blocking antibodies present; in others, there is no correlation.

Ragweed, grass, and tree pollen extracts polymerized with glutaraldehyde into huge molecular aggregates (MW ~ 20×10^6) have relatively few surface allergenic haptenic groups to react with IgE-sensitized mast cells (low allergenicity) but have many hidden antigenic groups which are exposed in degradation that stimulate large amounts of blocking antibodies (high antigenicity). These have been used successfully in clinical trials for immunotherapy and induce much higher blocking antibody titers and require fewer injections than with conventional aqueous extracts.

A second protective mechanism achieved by immunotherapy is the induction of IgE immune tolerance, in which IgE antibody production is suppressed by continued immunotherapy. If little or no IgE is produced, then continued sensitization of the mast cell does not occur. (See Chapter 24 for a discussion of the beneficial effects of allergen injections.)

In mice, allergens that have been partially denatured with mild urea treatment appear to preferentially stimulate IgE suppressor T cells, and this results in reduction of IgE antibodies. Mild denaturation of allergen during ether-glycerinated saline extraction or possibly glutaraldehyde treatment may act in a similar manner. Therefore, injections of denatured allergen (Fig 15–20) may preferentially stimulate IgE suppressor T cells to reestablish the normal damping mechanism for IgE. This is in contrast to the IgG blocking antibody system, in which helper T cells apparently are stimulated preferentially. New experimental immunotherapy methods are being proposed and tested that influence the regulatory controls of the IgE and IgG antibody systems, eg, D-glutamic acid-lysine (DGL)-ragweed, and polyethylene glycol-ragweed conjugates. These have successfully reduced IgE antibodies in mice and dogs and have diminished allergic responses in dogs. These methods are undergoing clinical trials in humans.

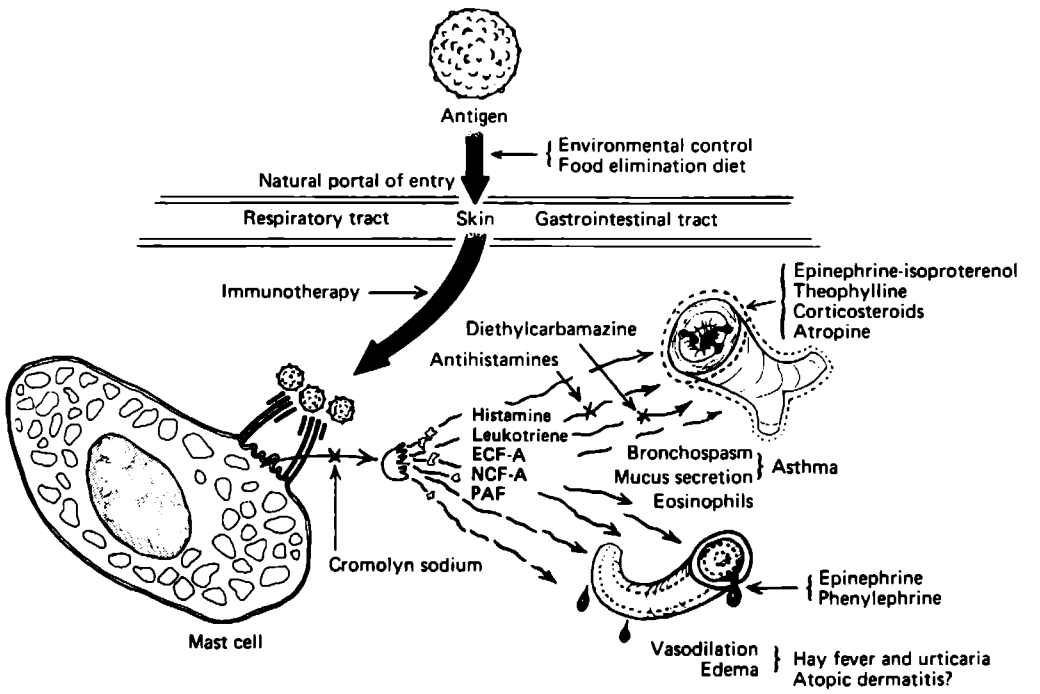

Figure 15–18. Therapeutic approaches to atopic reaction.

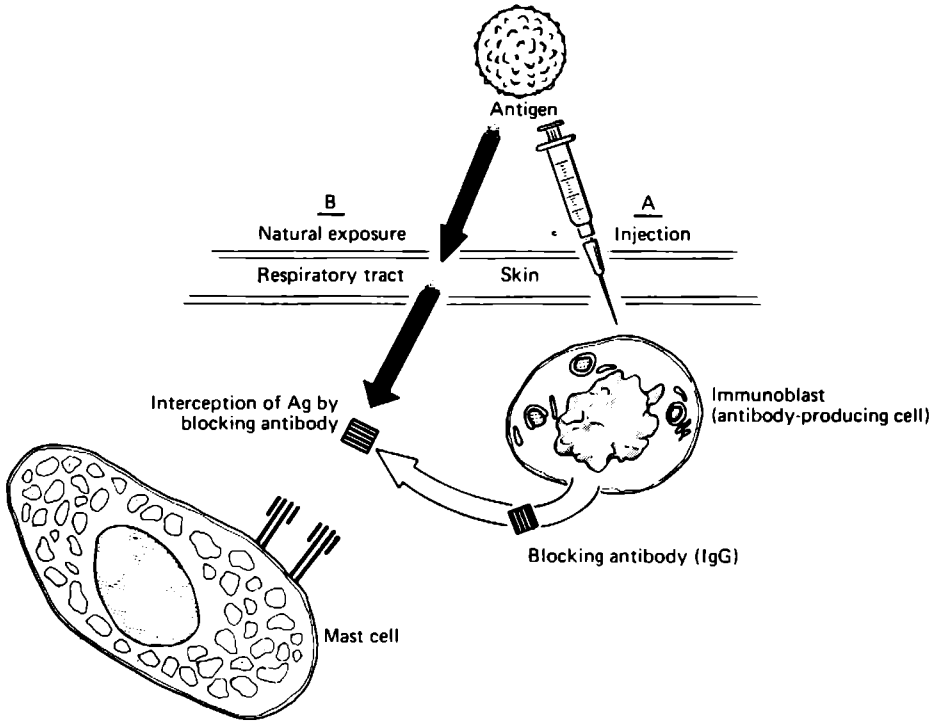

Figure 15–19. Immunization treatment.

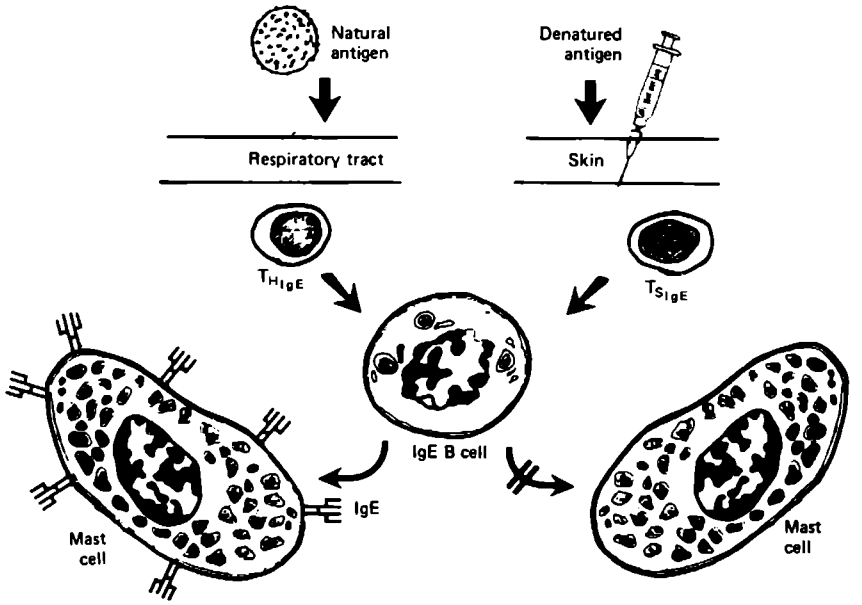

Figure 15–20. Immunotherapy stimulates IgE-specific suppressor T cells to abrogate IgE antibody production.

A third action of immunotherapy is nonspecific target cell desensitization, the mechanism of which is unknown. Children with both ragweed and *Alternaria* allergies, when treated with ragweed alone, had a decrease in the leukocyte histamine release with both ragweed and *Alternaria*. In fact, histamine release with anti-IgE antibodies was also dramatically reduced, indicating that the basophils were "desensitized." There are perhaps additional mechanisms by which immunotherapy works as well.

Immunotherapy with allergens in asthmatic patients was reported to be effective, especially in late-phase reactions. Warner reported on a double-blind placebo controlled study of 85 children with fairly severe perennial asthma, positive mite prick skin tests, and bronchial provocation with mite extract. Mite immunotherapy was 400 Noon units maximum every 8 weeks for 1 year. Symptom scores decreased significantly after 6 and 12 months, and pulmonary function improved in the actively treated group. No significant change occurred in the placebo group. Upon bronchoprovocation with mite extract after 1 year of immunotherapy, both groups still gave significant immediate responses with the same antigen dose. However, 11 out of 37 children who initially had late-phase reactions had lost these reactions and reported marked improvement in symptoms. This was the first report that immunotherapy improves late-phase reactions, with little change in immediate response. It was confirmed that high-dose *Alternaria* immunotherapy reduced severity of late, rather than early, asthmatic responses. Cutaneous late-phase reactions to ragweed were compared in both untreated and 3- to 5-year immunotherapy patients with ragweed allergy. Late-phase reactions occurred in 94% of untreated patients, whereas in the treated group, two-thirds had diminished or no late-phase reactions. These differences were highly significant at 4 and 8 hours after antigen injection. Size of cutaneous late-phase reactions correlated inversely with amount of IgG (but not IgE) antiragweed antibodies. Cutaneous late-phase reactions were suppressed in proportion to the amount of IgG antibody present. These studies suggest that efficacy of immunotherapy (and thus its usefulness in management of chronic asthma) is related to its effect on late-phase reactions and reduction in airway hyperreactivity.

The next area of allergy therapy is the enzyme cascade leading to release of histamine from mast cells, with attempts to stabilize lysosomal membranes in the mast cell. Cromolyn sodium acts by preventing the release of mediators from mast cell granules, probably by stabilizing lysosomal membranes. It does not prevent the antigen-antibody reaction. Previously it had been suggested that glucocorticoids stabilize lysosomal membranes; whether this actually occurs in humans is still under investigation.

Another action of corticosteroids is the inhibition of histidine decarboxylase, an enzyme that converts histidine into histamine. In guinea pigs, repeated treatment with compound 48/80 causes mast cells to lose their histamine content. In the presence of corticosteroids, mast cells fail to reaccumulate histamine until corticosteroids are withdrawn. An additional action of corticosteroids may be stimulation of suppressive factor of allergy (SFA), which turns off IgE antibody production.

The next area of therapeutic attack is interference with the action of the mediators of allergy. Prior administration of antihistamines, which resemble histamine in chemical structure, blocks histamine receptors on shock organ cells. When histamine is subsequently released, the receptors are occupied, and histamine is unable to act and is promptly destroyed by monoamine oxidases. Diethylcarbamazine and FPL 55712 appear to have a similar action for SRS-A. A diet high in oily fish (such as menhaden) that have high eicosapentaenoic acid precursors converts active LTC_4 into less active LTC_5 and lowers cyclooxygenase products, with potential lessening of asthma.

Finally, one can attempt to treat allergic diseases by counteracting the effects of the allergic reaction on the shock organ. As outlined above, biochemical control of mediator release and smooth muscle contraction can be counteracted both by appropriate blocking agents (atropine to block the cholinergics, phentolamine to block the α-adrenergics) and by reinforcement of the β-adrenergic system with isoproterenol, exogenous epinephrine, theophylline, and corticosteroids.

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16 Immunomodulation

William E. Seaman, MD

Much of this book describes the mechanisms by which the immune system is normally regulated. This chapter is a review of some of the means by which the immune response can be either enhanced or inhibited, with particular regard to clinical applications and to the use of monoclonal antibodies as specific agents for immune regulation.

NORMAL MECHANISMS FOR IMMUNE REGULATION

The immune system is self-regulatory. The response to antigen is specific and finite. If the antigen is removed, the immune response declines; but it establishes memory for the antigen, so that subsequent exposure to the same antigen is met with a more vigorous immune response. Normally, there is no response to self antigens; each individual has immunologic "tolerance" to its own antigens.

The development of agents that alter these processes of immune regulation has been advanced by an understanding of the cellular and molecular mechanisms by which the immune system is normally regulated. These mechanisms are reviewed in Chapter 7; they are summarized here in order to call attention to steps in the immune response that could be stimulated or suppressed.

Fig 16–1 presents a simplified scheme of the requirements for antibody production. Antibody is produced by **B lymphocytes.** The primary signal for antibody production is recognition of the antigen by the B cell surface immunoglobulin (IgM or IgD). This is influenced by the nature of the antigen as well as the route of administration. The response of B cells to antigen can be mimicked in vitro by antibody to IgM or IgD on the B cell surface. This stimulation causes resting B cells (B cells in G_0) to increase their cytoplasmic volume, to enter the early G_1 phase of cell cycling, and to become more sensitive to other signals for cell activation. These signals include B cell growth factors (BCGF) and B cell differentiation factors (BCDF) that are produced by **T lymphocytes.** T cell signals for B cell activation are provided by "helper/inducer" T lymphocytes (helper T cells), and the promotion of B cell activation by helper T cells is (under most conditions) promoted by contact between B cells and helper T cells. It is possible, however, to replace the requirement for helper T cells with soluble factors, so that a potential mechanism for immunopotentiation is the use of agents that directly activate B cells, particularly B cells that have been prestimulated by antigen. This strategy would be particularly useful in T cell immunodeficiency.

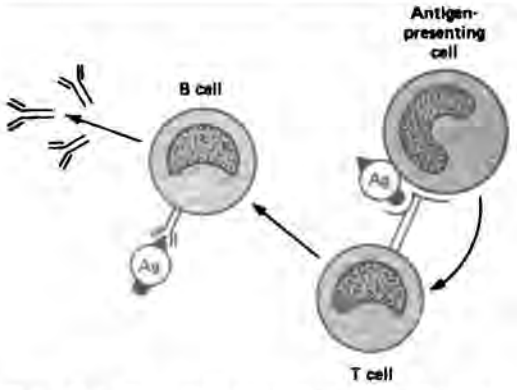


Figure 16–1. The cellular requirements for antibody production.

Helper T cells become activated to promote humoral immunity (and cellular immunity) when they encounter antigen on specialized **antigen-presenting cells (APC).** The requirements for T cell activation are similar to those for B cell activation. The primary signal is the recognition of antigen. T cells, however, must recognize antigen in association with self major histocompatibility antigens (MHA) on the APC. Just as B cells can be stimulated by antibody to surface antigen receptors (IgM or IgD), T cells can be stimulated by antibody to the T cell antigen receptor or to the structurally associated surface complex, T3 (also called CD3). As with B cells, the recognition of antigen by T cells is not alone sufficient for cell activation. Other signals potentiate the response, particularly interleukin-1 (IL-1). In the presence of both antigen and IL-1, T cells are activated to produce and to increase their receptors for IL-2, through which the response to antigen is greatly potentiated. Thus, the antigen-specific T cell response may be potentiated by "second signals" for T cell activation, such as the interleukins.

The antigen-specific response by T cells can also be potentiated by monoclonal antibodies that bind to T cell surface molecules that are distinct from the antigen receptor. Thus, it may be possible to stimulate T cells—and perhaps other cells of the immune sys-

tem—without the use of physiologic second signals but rather by monoclonal antibodies that mimic their effect.

Finally, because T cells are normally stimulated by antigen on antigen-presenting cells, a potential focus for altering immunity is the APC. This includes cells of the monocyte/macrophage lineage and, in particular, "dendritic" cells, which may be closely related to macrophages and which are potent stimulators of T cells.

IMMUNOPOTENTIATION

Clinically, immunopotentiation has been most effective in the prevention and treatment of infections. It has been less successful in treating cancer, and this remains a major area of clinical research. In immune deficiency, there is a need for treatment that will produce a generalized increase in immunity. This need for immunopotentiation has received increasing attention because of the spread of acquired immunodeficiency syndrome (AIDS).

Vaccination

The antigen specificity and memory of the immune system have been used clinically for almost 2 centuries to enhance the immune response to infectious agents by prior exposure to the agent. As examples, vaccination and other forms of immunization have eliminated—or greatly reduced in number—deaths from smallpox, poliomyelitis, pertussis, diphtheria, typhoid fever, mumps, and rubella. Vaccines now offer protection against infection with *Haemophilus influenzae*, *Streptococcus pneumoniae*, and hepatitis B virus. The ability to derive antigens for immunization by genetic engineering offers the promise of a rapid increase in vaccines, particularly for infectious agents that have been difficult to propagate in vivo or in vitro, such as *Mycobacterium leprae*, atypical mycobacteria, and malarial parasites.

Adjuvants

The response to immunization can be enhanced by a wide variety of agents, collectively referred to as adjuvants. Some adjuvants chiefly affect the way in which antigen is presented. For example, the immune response is increased when protein antigens are precipitated by alum or are presented on the surface of liposomes (membrane-bound vesicles). Emulsification of antigens prolongs the duration of antigen presentation.

Most adjuvants, however, act not on the antigen but on the host to increase the immune response. These adjuvants have received particular attention as potential agents of immunopotentiation when the antigen cannot be defined, as in the treatment of cancer, or where a broad increase in immunity is required, as in immune deficiencies. Until recently, the mechanisms by which such adjuvants promote immunity were not known. This remains true for many adjuvants, but for some the mechanism of immunostimulation is now partially understood. Moreover, the actions of physiologic stimulators of immunity, such as the interleukins, have been extensively studied in vitro, and these agents have been used pharmacologically as adjuvants.

A. Organic Adjuvants: The list of available adjuvants is long and is rapidly growing. They include a variety of organic molecules obtained from bacteria. For example, **muramyl dipeptide** (N-acetylmuramyl-L-alanyl-D-isoglutamine; MDP) is a bacterial peptidoglycan that conveys the adjuvant properties of mycobacteria, used in Freund's adjuvant. MDP can increase both humoral and cellular immunity. In vitro, humoral immunity is stimulated in the absence of mature T cells, suggesting that MDP may bypass the need for some of the factors provided by T helper cells in the generation of immunity. However, MDP also stimulates macrophages and, perhaps as a consequence, promotes the generation of helper T cells in vivo.

B. Synthetic Adjuvants: Synthetic adjuvants that increase host immunity include levamisole and isoprinosine. Levamisole is the levo isomer of tetramisole and, like tetramisole, was initially introduced as an anthelmintic agent. It potentiates humoral and cellular immunity through a mechanism that is T cell-dependent. Its usefulness as an adjuvant in the treatment of cancer has been validated in most clinical trials, but the results have not been sufficient to justify its widespread application for this purpose. Paradoxically, levamisole has been used with benefit in the treatment of rheumatoid arthritis, but for most patients the potential benefit is not offset by the risks, which include agranulocytosis. Inosiplex (Isoprinosine) is a complex containing inosine, the purine precursor of adenosine and guanosine. In vitro, isoprinosine promotes T cell mitogenesis. Clinically, isoprinosine reduces the recurrence rate of type 1 herpes simplex infections and appears to be beneficial in some other viral infections as well. Its usefulness as an adjuvant in the treatment of cancer has not been established.

C. Tuftsin: Tuftsin is a unique adjuvant that occurs naturally but has been synthesized. It is a 4-amino-acid peptide (threonine-lysine-proline-arginine) homologous to a sequence in the constant region of the immunoglobulin heavy chain. Tuftsin appears primarily to stimulate macrophages. Its occurrence in vivo suggests that it has a physiologic role in host defense.

D. Other Agents: Agents that have widespread effects on cellular metabolism will also affect immunity. These include inhibitors of prostaglandin synthesis or drugs that alter levels of intracellular cyclic nucleotides. These effects, however, have been inconstant. Experimentally, the alterations in immunity vary in part with the temporal relationship between the administration of drug and exposure to antigen. The immune system appears to be resilient to the chronic use of drugs that alter the metabolism of either prostaglandins (and other arachidonate metabolites) or

cyclic nucleotides; immunity is not significantly enhanced or suppressed. An important exception was described in some patients with lymphoma, in whom immunity was potentiated by the use of indomethacin, an inhibitor of prostaglandin synthesis. This did not, however, lead to regression of the tumors.

Lymphokines

Agents that act specifically on cells that are involved in the immune response offer the potential for selective immune stimulation. Two approaches to identifying such agents have been used. The first is the identification of physiologic "signals," such as the interleukins, that preferentially act on cells of the immune system. The second is the identification and stimulation of cell surface antigens that are uniquely expressed on cells of the immune system and that activate the cells on which they are expressed.

The **interleukins** are discussed in detail in Chapter 8, together with the **interferons.** Experimental and clinical trials have been advanced by the use of recombinant DNA to produce IL-1, IL-2, IFN γ, IFN β, and several variants of IFN α.

IFN α was the first of these lymphokines to be produced on a large scale by these methods. IFN α, as adjunctive therapy, is superior to placebo as assessed by a reduction in tumor size in a small but significant number of patients with lymphoma and, less often, certain other cancers. The effect of IFN α on cancer does not correlate well with changes in cellular immunity or natural killer cell activity. Although the regression of tumor may in part be due to the activation of host defenses, particularly by macrophages, it appears also that there is a direct effect of IFN α on the tumor cells. The use of IFN α causes flulike symptoms that can be severe, including fever, malaise, myalgias, and arthralgias. Clinical trials of recombinant IFN γ and IFN β are still in early stages.

Clinical trials have also been initiated with recombinant IL-2. From animal models, IL-2 appeared to be a candidate for the treatment of immunodeficiency, because it restores both humoral and cellular immunity in athymic (nude) mice. Early clinical trials in AIDS, however, have not shown dramatic success. IL-2 induces the production of IFN α by T lymphocytes, so that its clinical use is also associated with flulike symptoms. In addition, it causes marked fluid retention.

An alternative approach to the in vivo use of IL-2 has been the induction of **lymphokine-activated killer (LAK) cells** in vitro by incubation with IL-2. Under these conditions, lymphocytes acquire cytotoxicity against a broad range of tumor targets. Cells activated in this manner have been used to cause tumor regression in mice. In humans, LAK cells have been tested in conjunction with the systemic administration of recombinant IL-2. In a preliminary report of 25 patients with a variety of tumors, one patient with melanoma had remission of disease (10 months at the time of reporting) and 10 others had reduction in tumor size, mostly of pulmonary metastases. The long-term benefits of such therapy are unknown. These results, however, and other reports of tumor regression following treatment with lymphokines, warrant hope that potentiation of host defense may prove to be useful in tumor therapy.

Monoclonal Antibodies as Cell Activation Signals

Antibodies to either the T cell antigen receptor or to T3 cause activation signals presumed to be similar to those caused by the recognition of antigen (see Chapter 7). In the presence of monocytes, such antibodies are mitogenic. Moreover, monoclonal antibody to T3 stimulates killing by cytotoxic T cells. Thus, preincubation of T cells with anti-T3 increases nonspecific killing of tumor cells. The best targets, however, are tumors that bear Fc receptors. In this instance, the anti-T3 antibody binds to the Fc receptors on the tumor cells and activates cytolytic T cells by binding to the T3 surface molecule.

The activation of resting T cells by antibodies to T3 or to the T cell antigen receptor requires a "second signal." When T cells are stimulated by antibody to T3 in the presence of monocytes, the second signal can be provided by IL-1. Recent observations demonstrate that a second signal for T cell activation can also be provided by monoclonal antibodies to at least 2 distinct T cell surface molecules that are distinct from the IL-1 receptor. These are designated CD5, also called Leu-1 or Tp67 (MW 67,000), and Tp44 (MW 44,000). These antibodies, like IL-1, synergize with antibodies to the T3-antigen-receptor complex in promoting T cell activation. T cells can also be activated, in a manner that is independent of the T3-antigen-receptor complex, by monoclonal antibodies to CD2, a T cell surface molecule (MW 55,000) that binds to sheep red cells. Activation of T cells by monoclonal antibodies to CD2 requires 2 different antibodies. One antibody creates a conformational change in the CD2 molecule, allowing the second antibody to bind. Interestingly, antibody to a third site (epitope) on the CD2 molecule blocks the activation of T cells, indicating that a single surface molecule can provide signals for inactivation as well as activation of T cells.

Antibodies that potentiate the response of T cells to antigen have a potential for use as adjuvants. Such antibodies selectively bind to T cells and would, in theory, activate only those T cells that encounter antigen. This provides a mechanism for antigen-specific immune stimulation that does not require knowledge of the antigen.

The use of anti-T cell monoclonal antibodies to provide "second signals" for T cell activation in vivo requires that the antibodies not destroy the target cells. In mice, rat IgG2b monoclonal antibody to Lyt 1, a T cell antigen that is homologous to human CD5, destroys T cells and profoundly suppresses both humoral and cellular immunity. However, rat IgG2a monoclonal antibody to Lyt 1 does not efficiently kill T cells, and this antibody has been shown to prolong survival of tumor-bearing mice. Thus, different mono-

clonal antibodies to the same lymphocyte antigen may have different effects in vivo based on their ability to kill the target lymphocytes. The depletion of lymphocytes in vivo by antilymphocyte antibodies is dependent on the Fc portion of the antibody. This is discussed further in the section on immunosuppression. It will be seen that, with certain antibody isotypes or with $F(ab)'_2$ from antibody fragments (which lack the Fc portion), antilymphocyte antibodies can be used in vivo without depletion of the target cells. The "second signal" provided by antibody to CD5, the human homolog for Lyt 1, does not require an intact Fc fragment on the antibody. Consequently, it may be possible to achieve immunopotentiation in vivo with antilymphocyte antibodies that activate lymphocytes but do not deplete them.

Passive Immunization

An alternative to active immunization is "passive immunization," the administration of immune cells or of antibodies that are already reactive with a pathogen. The transfer of immune cells is also called adoptive immunotherapy.

The transfer of cellular immunity between animals from an inbred strain is easily accomplished. Restoring or increasing cellular immunity by the transfer of cells between histoincompatible humans is much more difficult, because of rejection of the cells by the host or because of graft-versus-host disease. Nonetheless, bone marrow grafting is used for the treatment of some immune deficiencies (see Chapter 20). As a treatment for AIDS, this has failed because of infection of the transplanted cells by the AIDS-related virus.

Because of problems associated with transferring foreign cells, there has been interest in transferring antigen-specific stimulators of cellular immunity from immune to nonimmune individuals. In 1955, H. S. Lawrence described the transfer of tuberculin-specific skin sensitivity, using an extract of leukocytes from sensitized donors. There have since been reports of successful treatment of a variety of immunodeficiency diseases with such **transfer factors.** Antigen-specific activity within the factors has been ascribed to nucleoproteins. Despite these successes, however, there have been failures, and controversy persists regarding the nature and use of transfer factors. This also applies to the clinical use of "immune RNA" obtained from lymphocytes from animals that have been immunized with a human tumor.

A more promising method for treatment with immune cells is the in vitro expansion and activation of lymphocytes from the host, with subsequent reinfusion of the cells. The nonspecific stimulation of killer cells in vitro by IL-2 has already been discussed as adjunctive therapy in the treatment of cancer. Exposure to tumor cells in vitro is also under investigation as a means of expanding tumor-specific T cells from the host prior to reinfusion. This has not been achieved in humans. In animal studies of the host defense against tumors, the in vitro expansion of helper T cells specific for tumor antigens has been more important than the expansion of cytotoxic T cells. The latter are presumably activated in vivo by the activated helper T cells.

A problem with all attempts to transfer or induce immunity against tumors has been the need to identify **tumor-specific antigens** that will induce an immune response. This issue is discussed in detail in Chapter 14. In animals in which tumors can be induced by viruses or by carcinogens, prior immunization with a tumor can protect against subsequent exposure to the same tumor. However, tumors that arise spontaneously are poorly immunogenic, and it has been difficult to define tumor-specific antigens on human tumors. Nonetheless, monoclonal antibodies have been used to identify antigens on a variety of human cancers, particularly melanomas and cancers of the colon or breast. These tumor antigens may be developmental antigens that are normally expressed on immature cells, but they are not expressed on most normal cells in the host and therefore may be useful targets for therapy.

The development of monoclonal antibodies against tumor antigens has rekindled interest in the clinical use of **passive immunization with antibodies.** Antisera against bacterial pathogens were used therapeutically earlier in this century, but their use was largely abandoned both because of problems with serum sickness (caused by a host response to the antiserum) and because antibiotics were developed that were effective against many of the common bacterial pathogens. Preparations of human immunoglobulin are still used clinically for treatment of a limited spectrum of infectious diseases, and immunoglobulin is the mainstay of therapy for patients with hypogammaglobulinemia (see Chapter 37).

The clinical use of monoclonal antibodies for passive immunization, like antisera, is also hindered by the potential for a host response to the antibody. Nonetheless, monoclonal antibodies have several advantages over antisera. First, they are highly specific. The development of monoclonal antibodies to pathogens or to tumor cells, as well as to normal cells, has allowed the characterization of antigens that had not previously been identified by antisera. Second, a monoclonal antibody is of one immunoglobulin isotype (class or subclass). Immunoglobulin isotypes differ in the biologic activity of their Fc portion with regard both to the activation of complement and to the interaction with Fc receptors on the surfaces of cells. Both of these functions are important considerations for the clinical use of monoclonal antibodies, as will be discussed. Third, monoclonal antibodies are reproducible reagents—in contrast to antisera, which are dependent on the host response of animals and on selective absorption, procedures that cannot be fully standardized. Monoclonal antibodies not only are uniform but can also be uniformly modified. As examples, the Fc portion can be removed or altered to prevent its biologic activity, or the antibody can be coupled to toxins in order to increase its cytotoxicity. Each of these features of monoclonal antibodies is im-

portant in considering their use as therapeutic agents in vivo.

When monoclonal antibodies are used for passive immunization, their unique specificity is a potential disadvantage. This may not allow antibody binding to the pathogen in amounts sufficient to allow removal of the pathogen. In this instance, either antisera or a combination of different monoclonal antibodies may be required.

The immunoglobulin class or subclass (isotype) of a monoclonal antibody is important if the antibody is to initiate a host response that will kill or eliminate an infection or a tumor. Antibodies elicit a host response by the activation of complement or by binding to cell surface Fc receptors (see Chapters 9 and 10). For human immunoglobulins, complement activation is initiated by IgG1, IgG2, IgG3, or IgM. Binding of immunoglobulins to Fc receptors is also restricted to certain immunoglobulin isotypes, but the restriction varies with the type of cell that bears Fc receptors.

Passive immunization with monoclonal antibody against tumors has been generally disappointing, with one important exception. Miller and coworkers successfully treated a patient with a B cell lymphoma by using a mouse monoclonal antibody against the idiotype of the immunoglobulin on the lymphoma. B cell lymphomas provide the single clear example of tumors with tumor-specific antigens. No cells other than B cells express immunoglobulin, and idiotypic determinants on the lymphoma will be expressed by only a small fraction of normal B cells. Treatment with a mouse IgG2a monoclonal antibody to such an idiotype was successful in the treatment of one patient with B cell lymphoma. In other patients, however, treatment with monoclonal anti-idiotype antibodies has not led to remission of disease. In some patients, the loss of efficacy was due to a change in idiotype of the tumor. Despite the treatment failures, the one success demonstrates the potential for passive immunization against a tumor.

The mechanisms by which B cell lymphoma cells are killed by monoclonal anti-idiotype antibody are not known. For normal lymphocytes, removal by antilymphocyte antibodies is primarily mediated by the mononuclear phagocyte system in the spleen. Antibody-coated cells are removed, when they circulate, by binding to Fc receptors and to C3b receptors on phagocytic cells. Thus, antibodies that fix complement are most effective in removing cells. This includes mouse IgG2a antibodies, the subclass of monoclonal antibody used successfully to treat B cell lymphoma.

Fig 16–2 reviews some of the mechanisms by which monoclonal antibodies might be used to elicit host defense against cancer. This includes not only antibodies reactive with tumor antigens but also antibodies that activate host cells, as discussed earlier.

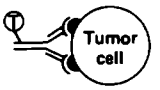
IMMUNOSUPPRESSION

Immunosuppression is particularly relevant to the treatment of graft rejection, autoimmunity, and allergy. For the clinical use of immune suppression, it would be desirable to restrict suppression to a limited range of antigens—eg, the antigens on an organ transplant—without suppressing the remainder of the host response. In the current treatment of autoimmunity or of graft rejection, the use of immunosuppression is not antigen-specific. The treatment of pollen allergies by repeated exposure to the allergen appears to induce tolerance to the allergen. In contrast, for allergy to venoms, repeated antigen challenge is associated with a change in the class of the immunoglobulin response from IgE to IgG.

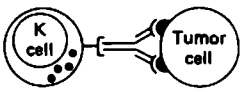
Cytotoxic Agents

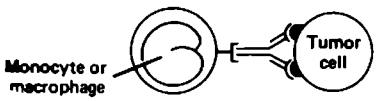
Cytotoxic agents such as cyclophosphamide, chlorambucil, azathioprine, and methotrexate block cell replication and preferentially kill dividing cells. **Cyclophosphamide** and **chlorambucil** alkylate DNA in both dividing and resting cells, leading to cell death during the mitotic phase of cell division. **Azathioprine** and **methotrexate** block DNA synthesis, preferentially killing cells that are in the S (DNA-synthesis) phase of the cell cycle at the time of treatment. The major use of these and other cytotoxic agents has been to kill malignant cells. Cytotoxic agents, however, also suppress both humoral and cellular immunity. Because B cells or T cells that respond to antigen are stimulated to divide, cytotoxic agents kill responding lymphocytes; but this is probably not the only mechanism for their immunosuppressive effects. Cytotoxic agents have been an important part of immunosuppressive therapy to facilitate organ transplantation (see Chapter 23). There is evidence from uncontrolled trials that cytotoxic agents, particularly cyclophosphamide, are effective in the treatment of Wegener's granulomatosis, polyarteritis nodosa, and other vasculitides. The efficacy of cytotoxic agents in the treatment of rheumatoid arthritis or systemic lupus erythematosus (SLE) appears to be less substantial. In the treatment of renal disease in patients with SLE, reduction of the number of deaths from renal failure is at least partially offset by deaths due to complications of therapy. Immunosuppression by cyclophosphamide is accompanied not only by an increase in the risk of infection but also by alopecia, gonadal dysgenesis, hemorrhagic cystitis, and an increase in cancer risk. Azathioprine, chlorambucil, and methotrexate have fewer side effects but are nonetheless toxic. In the treatment of autoimmunity, methotrexate has been the object of renewed interest in the 1980s in part because—as is not true of other cytotoxic agents—its use has not been associated with an increased incidence of cancer. Its extensive use in the treatment of psoriasis, however, has demonstrated its toxicity, particularly hepatotoxicity.

A: Complement-mediated cytolysis. Antibodies that activate complement (C) have the potential for killing target cells by formation of the complement membrane attack complex (C5b6789), causing membrane leakage. In practice this mechanism is not efficient for destroying nucleated cells in vivo.

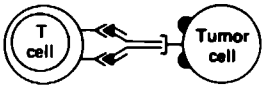


B: Cytolysis by an antibody conjugated with a toxin (T). Antibody-mediated cell killing can be increased by several orders of magnitude if the antibody is conjugated to one of the 2 chains (the α chain) of ricin toxin or diphtheria toxin. If the antibody recognizes a cell surface antigen and is then internalized by the cell, the chain kills the cell by interrupting protein synthesis. The use of $F(ab)'_2$ fragments instead of intact antibody reduces nonspecific uptake of antibody by cells that bear Fc receptors. In the place of plant toxins, the antibody may be conjugated to a cytotoxic drug or a source of radiation. A variation of this technique is to place the antibody on the surface of liposomes that contain a cytotoxic drug. The liposomes thereby preferentially bind to target cells. Each of these techniques has had success in animal tumors.

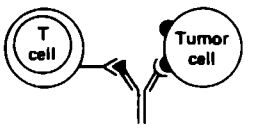




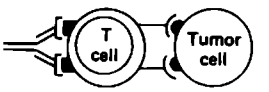
C and D: Antibody-dependent cell-mediated cytotoxicity (ADCC). Large granular lymphocytes, here called K cells *(C)*, and cells of monocyte-macrophage lineage *(D)*, express Fc receptors that allow them to bind and kill antibody-coated target cells. Killing of antibody-coated cells occurs most efficiently when the cells pass through the spleen. Killing does not require activation of the complement membrane attack complex, but it is greatly facilitated by antibodies that activate complement. This is probably because phagocytosis and killing by macrophages are promoted by the recognition of C3b on the target cell.



E: "Reverse ADCC." Tumors that express Fc receptors will bind monoclonal antibodies of the appropriate isotype. In vitro, this has been used to initiate T cell killing of tumor cells by using an antibody against the T cell antigen T3. This antibody not only will bind the T cells to the tumor but will also, in the presence of IL-1, activate T cells. The activation of cytolytic T cells leads to tumor lysis. In vitro studies have shown that T cell activation by monoclonal antibody to T3 will activate nonspecific killing of tumors that lack Fc receptors, but killing is more effective when T cells are bound to targets by this method. This is one instance in which monoclonal antibody could promote killing of tumors that lack a tumor-specific antigen. However, the method has the potential for killing any cells that express Fc receptors. More significantly, host cells with Fc receptors will kill the antibody-coated T cells. The demonstration of "reverse ADCC" by T cells is therefore of interest less for its practical use than for its demonstration that T cells can be activated by this mechanism to kill tumors.



F: T cell ADCC using a hybrid antibody. An alternative to "reverse ADCC" that would allow greater specificity is the use of a "hybrid" antibody that binds to both T3, on the T cell, and to a tumor-specific antigen. Such antibodies do not occur naturally, but they can be produced and have been shown to mediate T cell killing of tumors in vitro.



G: Activation of T cell killing by antibody to T cell "second signal" receptors. In this instance, the antibody does not bind to the tumor at all. Instead, it binds to a T cell surface molecule that promotes T cell activation. Several such antibodies have been described that activate T cells only in conjunction with a signal from the T cell antigen receptor or its associated structure, T3. Such antibodies would not activate all T cells, only those that encounter antigen. The activation signal does not require the Fc portion of immunoglobulin, so the antibodies need not destroy T cells (see text).

Figure 16–2. Mechanisms by which monoclonal antibodies might be used therapeutically in host defense against cancer. In each example, the tumor cell is assumed to express a unique antigen that can be recognized by the antibody (see Chapter 14). These tumor-specific antigens are represented diagrammatically as dark semicircles on the tumor.

Glucocorticoids

Glucocorticoids are used clinically for both their immunosuppressive and their anti-inflammatory effects. They are used regularly in the treatment of graft rejection and in severe asthma and autoimmunity. The immunosuppressive effects of glucocorticoids are poorly understood. Glucocorticoids reduce circulating lymphocytes and monocytes and suppress the production of IL-1 and IL-2. However, they have a diversity of other effects, and the clinical benefits of immunosuppression by glucocorticoids are often difficult to separate from their other actions, including anti-inflammatory effects. For example, the rapid benefit from glucocorticoids in patients with autoimmune thrombocytopenia cannot be ascribed to immune suppression. Glucocorticoids instead appear to reduce the clearance of antibody-coated platelets and possibly the binding of autoantibodies to platelets.

The chronic use of glucocorticoids causes frequent and sometimes severe adverse effects, including susceptibility to infection, osteopenia with bone fractures, diabetes, and cataracts. Some of the side effects can be reduced or eliminated by administering a single dose every 2 days. This is often insufficient, however, for the desired anti-inflammatory effects. Less frequent therapy with very large parenteral doses of glucocorticoids has been attempted in the treatment of autoimmune disease without clear advantage.

Cyclosporine

The most promising new immunosuppressive agent is cyclosporine, a cyclic polypeptide from soil fungi. Cyclosporine blocks T cell help for both humoral and cellular immunity, including suppression of IL-2 production. It does not block the activation of antigen-specific suppressor T cells, which may contribute to the development of antigen-specific tolerance following its use. In animals, cyclosporine has allowed permanent engraftment of histoincompatible transplants. The clinical benefits have been less dramatic in humans but are nonetheless significant for a variety of organ grafts as well as in the treatment of graft-versus-host disease.

The side effects of cyclosporine are also considerable—particularly renal failure and hepatotoxicity. The use of cyclosporine is associated with an increase in B cell lymphomas, although the contribution of other factors to this side effect is uncertain. In some cases of B cell lymphoma, the tumor has regressed upon reduction of immunosuppressive therapy. This supports a role for immune surveillance in protection against B cell lymphomas. The use of cyclosporine in the treatment of autoimmunity is under investigation.

Antilymphocyte Antibodies

Because of the desire to selectively alter lymphocyte function without affecting other cells, antisera against human lymphocytes or thymocytes have been produced in animals. For over 30 years, the immunoglobulins from these antisera have been used to treat patients, particularly recipients of organ grafts. Antithymocyte globulins have been shown to deplete lymphocytes and produce immunosuppression. There is evidence that they prolong graft survival in recipients of transplants, although the long-term benefit of such therapy, independent of the effects of other therapies, has been variable (see Chapter 23). This may be due in part to variations in the preparations of antiglobulins.

The development of monoclonal antibodies has allowed reproducibility in preparations of antibodies to lymphocytes. It is equally important that monoclonal antibodies have been used to identify new lymphocyte antigens that define functional subpopulations of lymphocytes and allow their selective depletion in vivo. All human T cells, for example, can be identified by the T3 (CD3) surface antigen. Monoclonal antibody to T3 has been used with limited success in treating host rejection of allografts. The clinical use of antibody to T3 has been complicated by several problems. First, like most antilymphocyte antibodies, mouse antibody to T3 is immunogenic, and the host response to the antibody reduces its efficacy. Second, antibody to T3 causes systemic effects (flushing, hypotension) that have been attributed to the rapid death of many T cells. An alternative explanation, however, may be suggested by the observation that antibody to T3 is mitogenic to T cells. Thus, some or all of the systemic response may represent activation of T cells, causing the synthesis and release of interferon, IL-2, or other T cell factors that have systemic effects. Third, 2 patients with graft-versus-host disease that were treated with a monoclonal antibody to T3 developed widespread and fatal proliferation of B cells that were infected with Epstein-Barr virus. This has not been reported in recipients of grafts other than bone marrow. Fourth, although the use of anti-T3 antibody appears to be immunosuppressive, it does not induce tolerance to grafts, so that its value so far has been limited to short-term inhibition of graft rejection.

In addition to the T3 antigen, which is expressed on all T lymphocytes, monoclonal antibodies have identified surface antigens that are expressed on subsets of T cells. These include the **T4 (CD4)** and **T8 (CD8) antigens,** which divide mature T cells into 2 mutually exclusive subsets (Fig 16–3A). Both of these T cell subsets recognize and respond to antigen, but the 2 subsets recognize antigen in association with different major histocompatibility antigens. For the most part, the two T cell subsets also subserve different functions (see Chapter 7). T cells that express T4 recognize antigen (on antigen-presenting cells) in association with class II major histocompatibility antigens (MHA II): HLA-DP, HLA-DQ, HLA-DR, and HLA-DZ. $T4^+$ cells are required for T cell help in the activation of B cells and are important in the induction of cellular immunity. $T4^+$ cells have therefore been called "helper/inducer" T cells. In vitro, antibodies to T4 prevent the response of $T4^+$ cells to antigen-presenting cells. They may also directly inhibit T cell function, even in the absence of antigen-presenting cells.

In contrast to $T4^+$ cells, $T8^+$ cells recognize anti-

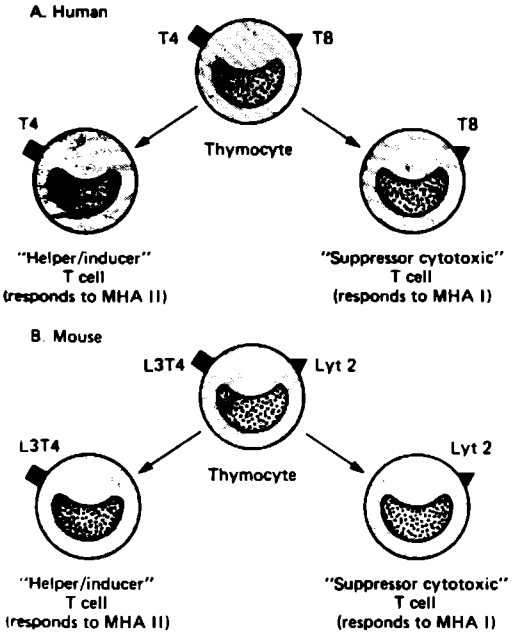


Figure 16–3. T cell differentiation antigens that distinguish functional T cell subsets. The human antigens T4 and T8 *(A)* are homologous to the murine antigens L3T4 and Lyt 2, respectively *(B)*.

gen in association with class I major histocompatibility antigens (MHA I): HLA-A, HLA-B, and HLA-C. $T8^+$ cells are the source of most cytotoxic T cells and mediate immune suppression. $T8^+$ cells have therefore been called "suppressor-cytotoxic " T cells. In vitro, antibodies to T8 prevent the response of $T8^+$ cells to antigen-presenting cells.

There is little clinical experience with antibodies to T4 or T8 in humans. In monkeys, which also express T4, antibodies to T4 have been used to prolong the survival of renal allografts. The grafts were eventually rejected, but the treatment was not sufficient to eliminate the $T4^+$ subpopulation, so it is possible that greater target cell depletion might have been more effective.

Mouse T lymphocytes express surface antigens that are similar in structure and in function to T4 and T8. In mice, the T4 homolog is called **L3T4,** and the T8 homolog is called **Lyt 2** (Fig 16–3B). Monoclonal antibodies to the mouse antigens have been used to study the effects on immunity in mice. The results of these studies are relevant to the potential use of antibodies to T4 or T8 in humans.

Treatment of mice with a rat IgG2b monoclonal antibody to L3T4 rapidly depletes $L3T4^+$ cells from the blood, lymph nodes, spleen, and Peyer's patches. This treatment profoundly suppresses humoral immunity, including suppression of immunity to the rat anti-L3T4 antibody, so that treatment with anti-L3T4 can be repeated for months without loss of efficacy and without evidence of serum sickness or anaphylaxis in the host. When treatment is stopped, the $L3T4^+$ cells recover unless the mouse has been thymectomized. It is not yet known whether depletion of $L3T4^+$ cells leads to immune tolerance, ie, whether exposure to antigen during depletion of $L3T4^+$ cells prevents the immune response to antigen after the $L3T4^+$ cells recover.

Treatment of mice with anti-L3T4 also depresses cellular immunity and prolongs graft survival. With a graft that is potently antigenic, such as skin, cellular immunity nonetheless eventually appears, and the graft is rejected. In these experiments, either cellular immunity can develop in the absence of $L3T4^+$ cells, or sufficient $L3T4^+$ cells escape destruction to promote cellular immunity. Studies are in progress in several laboratories to examine the survival of grafts that are less antigenic than skin.

Interestingly, depletion of $L3T4^+$ cells for as long as a month does not reduce the activity of natural killer cells, a subset of lymphocytes that spontaneously kills certain tumors.

Treatment of mice with antibody to $L3T4^+$ T cells not only profoundly suppresses humoral immunity and partially suppresses cellular immunity but also decreases autoimmune diseases. The effect of treatment with anti-L3T4 on humoral autoimmunity was examined in NZB/NZW F_1 mice, which spontaneously develop a disease with features of SLE, including antibodies to DNA and immune complex glomerulonephritis. In NZB/NZW female mice, anti-DNA antibodies begin to rise between the fourth and fifth months of life, and the animals die at a mean age of 7–9 months. Treatment of NZB/NZW mice with weekly injections of antibody to L3T4 at the onset of illness depletes $L3T4^+$ cells and prevents the development of autoimmunity. Treatment of mice after the onset of disease blocks the progression of disease and can even reverse established disease manifestations. Both anti-DNA antibodies and renal disease are reversed, and survival is prolonged.

Treatment of mice with antibody to L3T4 has also been used to interrupt a mouse model for cellular autoimmunity, **experimental allergic encephalomyelitis,** which can be induced in mice by immunization with myelin basic protein. There ensues a cellular immune response against myelin basic protein in the central nervous system, resulting in an illness that closely resembles multiple sclerosis. Treatment of mice with antibody to L3T4 at the time of immunization or early in the course of disease significantly reduces the expression of the disease. Anti-L3T4 is also effective in treating collagen-induced arthritis in mice.

These studies suggest that monoclonal antibodies to T4 could be used to suppress autoimmunity in humans, but the treatment would cause severe immune suppression. In order to reduce the risks of this suppression, it is possible that both autoimmunity and normal immunity could be interrupted by antibody to the T4 antigen without depleting the $T4^+$ cells. In vitro studies using cell lines indicate that antibodies to T4 or to L3T4 block the response of T cells that bear these antigens, under conditions where the antibodies do not

kill the cells. Thus, if similar conditions of binding could be achieved in vivo, without deletion of the target cells, it may be expected that T cell "help" would be interrupted. One means of approaching this is the use of monoclonal antibodies that are, because of their isotype, inefficient at clearing target cells. With prolonged therapy, however, even these antibodies eventually deplete target cells, so that other methods have also been considered. The $F(ab)'_2$ fragment of antibody is inefficient at depleting cells, because it lacks the Fc portion of immunoglobulin, which activates complement and is recognized by the mononuclear phagocyte system. The effects of $F(ab)'_2$ anti-L3T4 antibodies on immunity have not yet been assessed in vivo. Another approach may be to alter the Fc portion of immunoglobulin so that it is not recognized by the Fc receptors of the mononuclear phagocyte system. The ability to block autoimmunity by any of these methods would have the advantage of being rapidly reversible in the event of infection or other complications of immune suppression.

Antibodies to MHA

Like human T cells, mouse L3T4$^+$ cells are activated by the recognition of antigen in association with MHA II, and activation is interrupted by monoclonal antibodies or by antisera to MHA II. In mice, monoclonal antibodies to MHA II have been used to block both normal immunity and autoimmunity. Treatment of mice with antibody to MHA II at the time of immunization can produce long-term cell-mediated suppression of the cellular immune response to the immunogen; ie, immune tolerance is induced. This treatment has not yet been successfully used to induce long-term tolerance to organ grafts, but such studies are continuing.

The mechanisms by which monoclonal antibodies to MHA II inhibit the humoral immune response to antigen have not been established. B cells acquire MHA II early in their maturation, so treatment with antibodies to MHA II may block humoral immunity simply by depleting secretory B cells. Contrary evidence lies in the observation in F_1 mice that monoclonal antibody to MHA II from one parent blocks an immune response that is regulated by the target MHA II but that the antibody does not block an immune response regulated by MHA II from the other parent. Because B cells simultaneously express MHA II inherited from both parents, this effect cannot be explained by B cell depletion. Rather, anti-MHA II must specifically alter immune regulation. Curiously, the selective effect of anti-MHA II against one parental haplotype was seen only for immunization without adjuvant; antibody against one parental MHA II blocked responses restricted by either parental haplotype when antigen was presented in adjuvant. Monoclonal antibodies to MHA II have also been used to successfully treat autoimmunity in NZB/NZW mice. A monoclonal antibody to MHA II from the NZW parent (histocompatibility type H-2^z) was more effective than a monoclonal antibody to MHA II from the NZB parent (H-2^d). However, in these studies, the antibody to NZW MHA II (mouse Ig2a) more effectively eliminates target cells than the antibody to NZB MHA II (mouse IgG2b).

MHA II are expressed not only on mature B cells but also on monocytes, macrophages, dendritic cells, Langerhans cells, Kupffer cells, epidermal cells, and, in humans, activated T cells. In primates, MHA II are constitutively expressed on vascular endothelium, and they can be induced on these and other cells, such as thymic epithelial cells. This broad expression of MHA II may complicate the clinical use of antibodies to MHA II. The expression of MHA II on vascular endothelium may account for thrombotic complications following the use of antibodies to MHA II in primates. On the other hand, clinical trials have been initiated in Europe with placental sera for the treatment of autoimmune disease. Placental sera contain antibodies to MHA II and have so far been used without apparent thrombotic complications.

Another approach to the suppression of T cell immunity has been the use of monoclonal antibodies to T cell antigens that are expressed by activated T cells, with the intent of selectively deleting antigen-responding T cells. An example is the **receptor for IL-2** (Tac antigen, CD25), which is expressed in low density on resting T cells. Activation of T cells induces an increase in transcription of the messenger RNA for the IL-2 receptor and a subsequent increase in the density of the receptor on the cell suface. IL-2 itself promotes the expression of its receptor when T cells are stimulated. Antibodies to the IL-2 receptor have been tested in recipients of organ grafts with initial success, as assessed by prolonged graft survival.

In the treatment of autoimmunity, another approach has been to identify unique antigenic sites (**idiotypes**) on autoantibodies. Monoclonal antibodies to these idiotypes can be used to delete the autoantibodies and the cells that produce them. For example, a large proportion of the anti-DNA antibodies in both murine and human SLE are known to share a common idiotype. Monoclonal antibody to an idiotype on murine anti-DNA antibodies has been used to treat autoimmunity in NZB/NZW mice. This treatment transiently reduced the level of anti-DNA antibodies and the rate of renal failure, but the disease subsequently progressed because of an increase in anti-DNA antibodies that lacked the shared idiotype. It is possible that a broader range of anti-idiotype antibodies would be more effective—or that a combination of anti-idiotype therapy, together with other therapies to prevent the occurrence of new autoantibodies, would be useful in treatment. A cautionary note is struck, however, by the finding in one study that treatment with a monoclonal antibody to one idiotype on anti-DNA antibodies actually induced the production of anti-DNA antibodies bearing the idiotype.

SUMMARY

With the exception of immunization, most of our methods for immunostimulation or immunosuppression are not antigen-specific, and most have effects that are not limited to the immune response. It now appears possible that this can be changed. This expectation is based on (1) the identification of cell subsets that contribute to the immune response; (2) the isolation of molecules that serve as regulatory signals between these cells; (3) the characterization of cell surface structures that transmit intracellular signals leading to cell activation or inactivation; and (4) an understanding of the intracellular events that lead to cell activation. It is now possible to selectively deplete cell populations that participate in the immune response and, under some circumstances, to selectively activate them. The next major step is to develop methods for antigen-specific inactivation or suppression of cells. There has been preliminary success in this area. It is expected that clinical methods for specific immunosuppression and immunostimulation can be developed that will act selectively on the immune system and will alter immunity to selected antigens.

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Section II. Immunologic Laboratory Tests

Clinical Laboratory Methods for Detection of Antigens & Antibodies

17

Daniel P. Stites, MD, & R. P. Channing Rodgers, MD

One of the major challenges for modern medical science is the translation of basic advances in immunochemistry and immunobiology into diagnostic and therapeutic procedures that will be useful in the practice of clinical medicine. Much of this work is done in the clinical immunology laboratory, where tests that utilize a great many of the recently elucidated principles of basic immunology can be performed on a wide variety of samples taken from patients. The results of these laboratory procedures are then utilized by practicing physicians in the diagnosis and treatment of clinical disorders. Furthermore, qualitative and quantitative analysis of several features of the immune response has led to better understanding of the pathogenesis of many clinical disorders. This understanding in turn has stimulated further basic scientific research in immunology. In fact, observations made by clinical investigators in immunology have frequently dramatically changed the course of basic research in immunology and related fields. An example is the impetus given to research on T cell and B cell immune systems by careful clinical descriptions of patients with thymic aplasia and hypogammaglobulinemia.

In the past 2 decades, immunologic laboratory methods have gradually become increasingly more refined and simplified. Because of their inherent specificity and sensitivity, these methods have now achieved a central role in modern clinical laboratory science. The goals of laboratory medicine are to improve the availability, accuracy, and precision of a body of medically important laboratory tests, to ensure correct interpretation, and to assess the significance of new tests introduced into clinical medicine. With the marked proliferation of new laboratory tests employing immunologic principles, these methods of laboratory diagnosis have often been uncritically applied to clinical situations. A better understanding of the methods used in the immunology laboratory should provide the student and practitioner of medicine with a useful guide for correct application and interpretation of this body of knowledge.

In the present chapter, tests for the detection of antigens and antibodies in clinical practice are discussed. Most of the techniques described involve application in the clinical laboratory of the principles of immunochemistry discussed in detail in Section I. This chapter and the next one on cellular immunology are not meant to be comprehensive laboratory manuals. Rather, the principles of the various immunologic methods and their application to selected clinical problems are reviewed. It is hoped that careful study of these 2 chapters in conjunction with the first section of this book will provide the reader with a solid background for an enhanced understanding of the detailed discussions of clinical immunology and descriptions of specific tests used in various disorders presented in Section III.

The topics covered in this chapter include the following:

(1) Immunodiffusion
(2) Electrophoresis and immunoelectrophoresis
(3) Immunochemical and physicochemical methods
(4) Binder ligand assays
(5) Immunohistochemical techniques (immunofluorescence)
(6) Agglutination
(7) Complement function

IMMUNODIFFUSION

The purpose of all immunodiffusion techniques is to detect the reaction of antigen and antibody by the precipitation reaction. Although the formation of antigen-antibody complexes in a semisolid medium such as agar is dependent on buffer electrolytes, pH, and temperature, the most important determinants of the reaction are the relative concentrations of antigen and antibody. This relationship is depicted schematically in Fig 17–1. Maximal precipitation forms in the area of equivalence, with decreasing amounts in the zones of antigen excess or antibody excess. Thus, formation of precipitation lines in any immunodiffusion system is highly dependent on relative concentrations of antigen and antibody. The **prozone phenomenon** refers to suboptimal precipitation which occurs in the region of antibody excess. Thus, dilutions of antisera need to be reacted with fixed amounts of antigen in order to obtain maximum precipitin lines. The prozone phenomenon is a cause of misinterpretation of immunoelectrophoresis patterns in the diagnosis of paraproteinemias.

Immunoprecipitation is the simplest and most direct means of demonstrating antigen-antibody reac-

tions in the laboratory. The application of immunoprecipitation to the study of bacterial antigens launched the field of serology in the first part of the 20th century. In 1946, Oudin described a system of single diffusion of antigen and antibody in agar-filled tubes. This important advance was soon followed by Ouchterlony's classic description of double diffusion in agar layered on slides. This method is still in widespread use today and has many applications in the detection and analysis of precipitating antigen-antibody systems.

Immunodiffusion reactions may be classified as single or double. In single immunodiffusion, either antigen or antibody remains fixed and the other reactant is allowed to move and complex with it. In double immunodiffusion, both reactants are free to move toward each other and precipitate. Movement in either form of immunodiffusion may be linear or radial. Specific examples are discussed in the remainder of this section.

Immunodiffusion has an important clinical application in the quantitation of serum immunoglobulins. Quantitative analysis of serum proteins is often done by more sensitive and automated methods such as nephelometry, ELISA, or RIA. Single radial diffusion in agar has largely been supplanted by these methods.

METHODOLOGY & INTERPRETATION

Double Diffusion in Agar

This simple and extremely useful technique (also called **Ouchterlony analysis**) is based on the principle that antigen and antibody diffuse through a semisolid medium (eg, agar) and form stable immune complexes which then can be analyzed visually.

The test is performed by pouring molten agar onto glass slides or into Petri dishes and allowing it to harden. Small wells are punched out of the agar a few millimeters apart. Samples containing antigen and antibody are placed in opposing wells and allowed to diffuse toward one another in a moist chamber for 18–24 hours. The resultant precipitation lines that represent antigen-antibody complexes are analyzed visually in indirect light with the aid of a magnifying lens. When antigen and antibody are allowed to diffuse in a radial fashion, an arc which approximates a straight line is formed at the leading edges of the diffusing antigen and antibody. The types of patterns produced in simple double diffusion are shown in Fig 17–2.

Double diffusion is commonly performed by placing antigen and antibody wells at various angles for comparative purposes. The 3 basic characteristic patterns of those reactions are shown in Fig 17–3. In addition to these 3 basic patterns, more complex interrelationships may be seen between antigen and antibody. The formation of a single precipitation line between an antigen and its corresponding antiserum can be utilized as a rough estimation of antigen or antibody purity. However, the relative insensitivity of the test and the limitation of immunodiffusion to *precipitating* antigen-antibody reactions partly restrict the applications of this technique. It is most useful in demonstrating identity of serologic reactions to various infectious agents with human positive control antibodies.

Double immunodiffusion in agar can also be used for semiquantitative analysis in human serologic systems where the specificity of the precipitation lines has already been determined. Such an analysis is performed by placing antibody in a central well surrounded circumferentially by antigen wells (Fig 17–4). Serial dilutions of antigen are placed in the surrounding wells, and the development of precipitation lines can be taken as a rough measure of antigen concentration. Alternatively, this form of analysis is very useful in determining the approximate precipitating titer of an antiserum by simply reversing the location of antigen and antibody in the pattern (Fig 17–4).

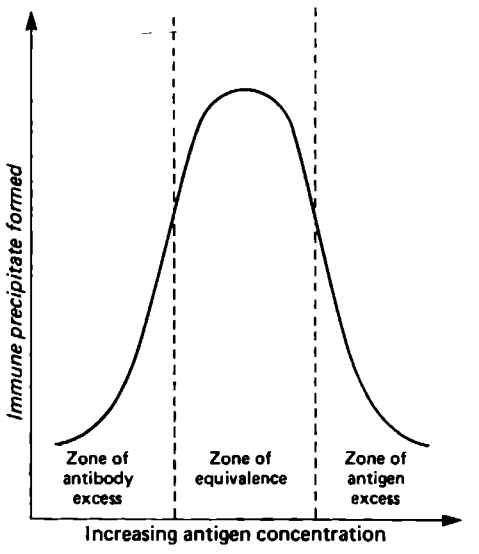


Figure 17–1. Antigen-antibody precipitin curve. Typical precipitin curve resulting from titration of increasing antigen concentration plotted against amount of immune precipitate formed. Amount of antibody is kept constant throughout.

Single Radial Diffusion

The double immunodiffusion system is only semi-

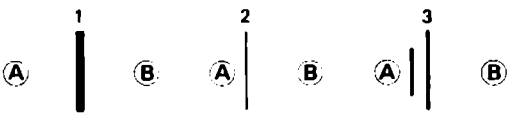


Figure 17–2. Reactions in simple double diffusion. In *(1)* antigen A and antibody B react equidistantly and intensely at equivalence. In *(2)* antigen A is present in reduced concentration or has not diffused as rapidly owing to size or charge, forming a precipitin line closer to the antigen well. In *(3)* a contaminant or impurity present in antigen A is reacting with antibody B.

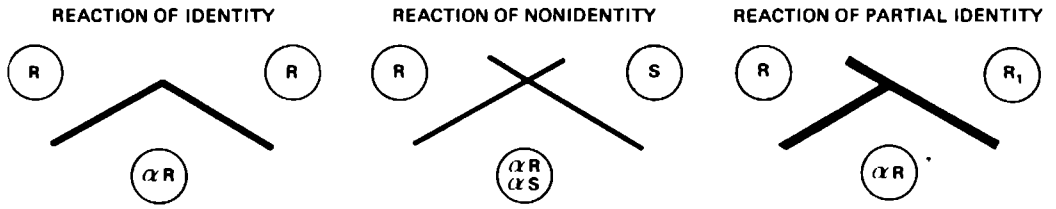


Figure 17–3. Reaction patterns in angular double immunodiffusion (Ouchterlony). R = antigen R, S = antigen S, R_1 = antigen R_1, αR = antibody to R, αS = antibody to S. **Reaction of identity:** Precisely similar precipitin lines have formed in the reaction of R with αR. Note that the lines intersect at a point. **Reaction of nonidentity:** Precipitin lines completely cross owing to separate interaction of αR with R and αS with S when R and S are non-cross-reacting antigens. **Reaction of partial identity:** αR reacts with both R and R_1 but forms lines that do not form a complete cross. Antigenic determinants are *partially* shared between R and R_1.

quantitative. In 1965, Mancini introduced a novel technique employing single diffusion for accurate quantitative determination of antigens. This technique grew out of the simple linear diffusion technique of Oudin by means of the incorporation of specific antibody into the agar plate. Radial diffusion is based on the principle that a quantitative relationship exists between the amount of antigen placed in a well cut in the agar-antibody plate and the resulting ring of precipitation. The technique is performed as diagrammed in Fig 17–5.

In the method described originally by Mancini, the *area* circumscribed by the precipitation ring was proportionate to the antigen concentration. This end point method requires that the precipitation rings reach the maximal possible size, which often requires 48–72 hours of diffusion. Alternatively, the single radial diffusion method of Fahey allows measurement of the rings prior to full development. In this modification, the logarithm of the antigen concentration is proportionate to the *diameter* of the ring.

A standard curve is experimentally determined with known antigen standards, and the equation which describes this curve can then be used for the determination of antigen concentration corresponding to any diameter size (Fig 17–6). The sensitivity of these methods is in the range of 1–3 μg/mL of antigen.

An important clinical application of single radial diffusion is in the measurement of serum proteins—for example, immunoglobulin concentrations. A monospecific antiserum directed only at Fc or H chain determinants of the immunoglobulin molecule must be

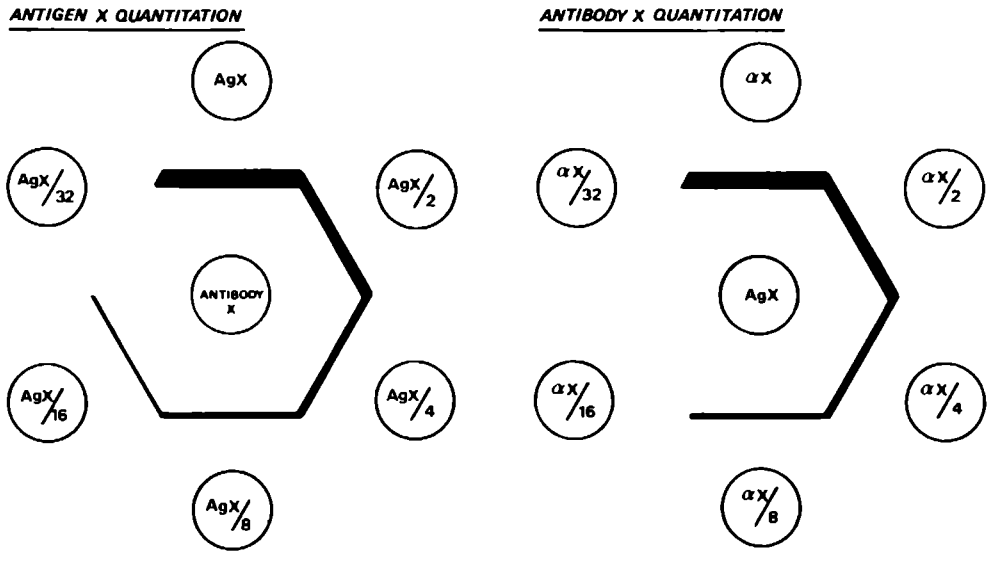


Figure 17–4. Semiquantitative analysis of antigen and antibody by double immunodiffusion. Antigen X is serially diluted and placed circumferentially in wells surrounding the central well containing antibody against antigen X. Precipitin lines form with decreasing thickness until no longer visible at dilution of 1:32 of antigen X. On the right, a similar pattern is generated but with serial 2-fold dilutions of antibody X. Formation of a single precipitin line indicates that a single antigen-antibody reaction has occurred.

incorporated into the agar in order to determine immunoglobulin concentrations, since L (light) chain determinants are shared among immunoglobulin classes. Owing to the relatively low concentrations of IgD and IgE in human serum, this technique is used primarily to determine the other 3 immunoglobulin classes, IgG, IgA, and IgM. However, by decreasing the amount of specific anti-immunoglobulin antiserum placed in the agar, so-called "low level" plates can be produced that have increased sensitivity for detection of reduced levels of serum immunoglobulins (IgG, IgA, IgM, and IgD).

There are a number of common pitfalls in the interpretation of single radial diffusion tests for immunoglobulin quantitation: (1) Polymeric forms of immunoglobulin such as occur in multiple myeloma or Waldenström's macroglobulinemia diffuse more slowly than native monomers, resulting in underestimation of immunoglobulin concentrations in these diseases. (2) High-molecular-weight immune complexes that may circulate in cryoglobulinemia or rheumatoid arthritis will result in falsely low values by a similar mechanism. (3) Low-molecular-weight forms such as 7S IgM in sera of patients with macroglobulinemia, systemic lupus erythematosus, rheumatoid arthritis, and ataxia-telangiectasia may give falsely high values. This phenomenon results from the fact that 7S IgM diffuses more rapidly than the 19S IgM parent molecule, which is used as the standard. (4) Reversed precipitation may occur in situations where the test human serum contains anti-immunoglobulin antibodies. In such a circumstance, diffusion and precipitation occur in 2 directions simultaneously and may result in falsely high values. This phenomenon has been well documented in the case of subjects with IgA deficiency who have antibodies to ruminant proteins in their serum. The problem of IgA quantitation in this circumstance can be avoided by using anti-immunoglobulin from rabbits (ie, a nonruminant species).

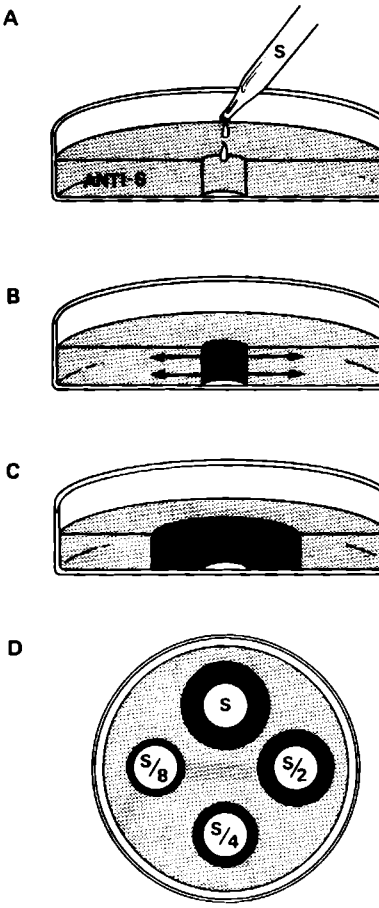


Figure 17–5. Single radial diffusion in agar (radial immunodiffusion). *A:* Petri dish is filled with semisolid agar solution containing antibody to antigen S. After agar hardens, the center well is filled with a precisely measured amount of material containing antigen S. *B:* Antigen S is allowed to diffuse radially from the center well for 24–48 hours. *C:* Where antigen S meets corresponding antibody to S in the agar, precipitation results. After reaction proceeds to completion or at a timed interval, a sharp border or a ring is formed. *D:* By serial dilution of a known standard quantity of antigen S—S/1, S/2, S/4, S/8—rings of progressively decreasing size are formed. The amount of antigen S in unknown specimens can be calculated and compared with standard in the timed interval (Fahey) method (Fig 17–6).

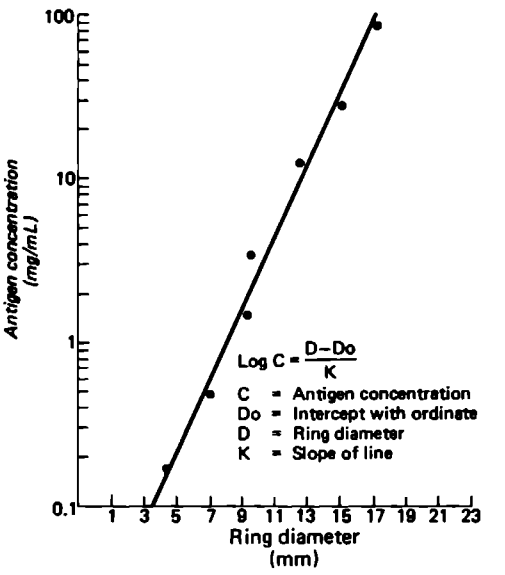


Figure 17–6. Standard curve for single radial diffusion. Relationship between ring diameter and antigen concentration is described by the line constructed from known amounts of antigen (Fig 17–5). Equation and curve for timed interval (Fahey) method.

APPLICATIONS: SERUM IMMUNOGLOBULIN LEVELS IN HEALTH & DISEASE

Serum immunoglobulin levels are dependent on a variety of developmental, genetic, and environmental factors. These include ethnic background, age, sex, history of allergies or recurrent infections, and geographic factors (eg, endemic infestation with parasites results in elevated IgE levels). The patient's age is especially important in the interpretation of immunoglobulin levels. Normal human infants are born with very low levels of serum immunoglobulins which they have synthesized; the entire IgG portion of cord serum has been transferred transplacentally from the mother (Fig 17–7). If an in utero infection occurs, cord IgM and IgA are elevated. After birth, this IgG decays, resulting in a falling serum IgG level. This trend is reversed with the onset of significant autologous IgG synthesis. There is a gradual and progressive increase in IgG, IgA, and IgM levels until late adolescence, when nearly normal adult levels are achieved (Fig 17–8). Furthermore, it is clear that there is a great deal of variability in immunoglobulin levels in the normal population (Fig 17–8 and Table 17–1).

In routine clinical laboratory practice, only IgG, IgA, IgM, and IgE levels are ordinarily measured. Abnormalities of serum IgD concentrations have not clearly been associated with specific disease states. In fact, this immunoglobulin is the major B cell receptor and may play only a minor role as a circulating antibody. IgE levels, on the other hand, are useful in differential diagnosis of allergic (see Chapters 15 and 24), parasitic, and rare immunodeficiency states (Chapter 20). Measurement of serum IgE levels requires sensitive methods such as RIA or enzyme-linked immunoassay.

Individual changes in serum immunoglobulins have been recorded in many diseases. A partial list of the instances of quantitative abnormalities in immunoglobulins is listed (Table 17–2). For a detailed discussion of immunoglobulin disorders, the reader is referred to Chapter 20.

ELECTROPHORESIS

Analysis of the heterogeneity in individual proteins is most readily accomplished by electrophoresis. The separation of proteins in an electrical field was perfected in 1937 by Tiselius, who used free or moving boundary electrophoresis. However, owing to the relative complexity of this method, zone electrophoresis in a stabilizing medium such as paper or cellulose acetate has replaced free electrophoresis for clinical use.

In 1952, a 2-stage method was reported that combined electrophoresis with immunodiffusion for the detection of tetanus toxoid by antiserum. Shortly thereafter, the now classic method of immunoelectrophoresis (IEP) was introduced by Williams and Grabar and by Poulik. In this technique, both electrophoresis and double immunodiffusion are performed on the same agar-coated slide. During the past 35 years, immunoelectrophoresis has become the cornerstone of clinical paraprotein analysis as well as a standard method for immunochemical analysis of a

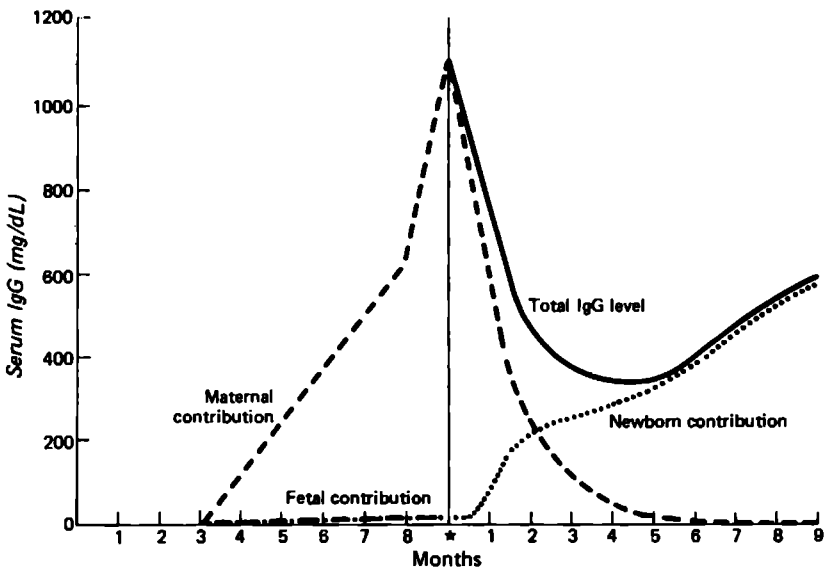


Figure 17–7. Development of IgG levels with age. Relationship of development of normal serum levels of IgG during feta and newborn stages and maternal contribution. (Modified from Allansmith M et al: *J Pediatr* 1968;72:289.)

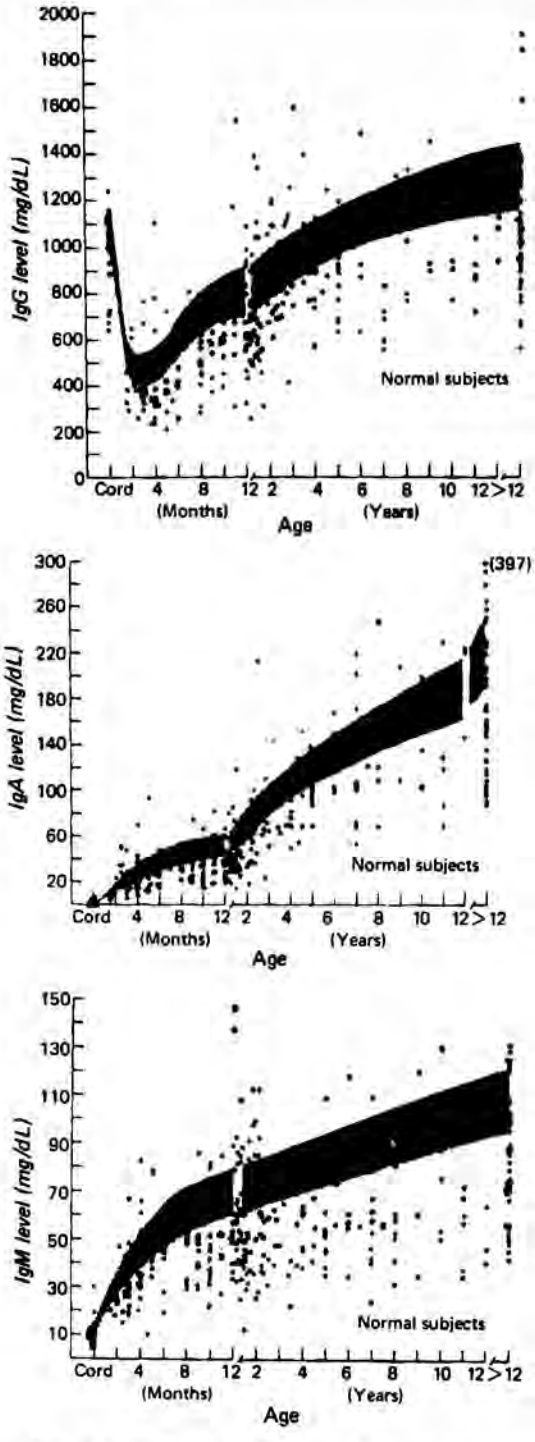


Figure 17–8. An example of variation of normal subjects serum levels of IgG, IgA, and IgM with age. Scattergrams of levels of IgG, IgA, and IgM in normal subjects. Shaded areas are ±1 SD of the mean; each point represents one subject. (From Stiehm ER, Fudenberg HH: *Pediatrics* 1966;37:718.)

wide variety of proteins. More recently, immunofixation electrophoresis and electroimmunodiffusion methods have been introduced. Various electrophoretic methods and examples of their uses in clinical immunodiagnosis are described in the following paragraphs.

ZONE ELECTROPHORESIS

Proteins are separated in zone electrophoresis almost exclusively on the basis of their surface charge (Fig 17–9). The supporting medium is theoretically inert and does not impede or enhance the flow of molecules in the electrical field. Generally, paper, agarose, or cellulose acetate strips are employed as supporting media. However, a major advantage of cellulose acetate is the speed of completion of electrophoretic migration (ie, 60–90 minutes compared to hours for paper). Additionally, cellulose acetate is optically clear; microquantities of proteins may be applied; and it is adaptable to histochemical staining procedures. For these reasons, cellulose acetate or agarose is commonly used as the supporting medium for clinical zone electrophoresis.

In the technique itself, serum or other biologic fluid samples are placed at the origin and separated by electrophoresis for about 90 minutes, using alkaline buffer solutions. The strips are then stained for protein and scanned in a densitometer. In the densitometer, the stained strip is passed through a light beam. Variable absorption due to different serum protein concentrations is detected by a photoelectric cell and reproduced by an analog recorder as a tracing (Fig 17–9). Scanning converts the band pattern into peaks and allows for quantitation of the major peaks. Normal human serum is separated into 5 major electrophoretic bands by this method, ie, albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin.

Applications

Zone electrophoresis is extremely valuable in the diagnosis of human paraprotein disorders such as multiple myeloma and Waldenström's macroglobulinemia (Fig 17–10). In these disorders, an electrophoretically restricted protein spike usually occurs in the γ-globulin region of the electrophoretogram. Since in zone electrophoresis the trailing edge of immunoglobulins extends into the β region and occasionally the α region, spikes in these regions are also consistent with paraproteinemic disorders involving immunoglobulins.

A marked decrease in serum γ-globulin concentration such as occurs in hypogammaglobulinemia can also be detected with this technique (Fig 17–10). Reduction in IgA or IgM to very low levels cannot be detected by this method, since they represent such a relatively small fraction of total immunoglobulins. Free light chains are readily detectable in urine when present in increased amounts such as in Bence Jones proteinuria of myeloma (Fig 17–11). Zone elec-

Table 17–1. Examples of levels of immune globulins in serum of normal subjects at different ages.*

Age	Number of Subjects	Level of IgG†		Level of IgM†		Level of IgA†		Level of Total γ-Globulin†	
		mg/dL (Range)	% of Adult Level	mg/dL (Range)	% of Adult Level	mg/dL (Range)	% of Adult Level	mg/dL (Range)	% of Adult Level
Newborn	22	1031 ± 200 (645–1244)	89 ± 17	11 ± 5 (5–30)	11 ± 5	2 ± 3 (0–11)	1 ± 2	1044 ± 201 (660–1439)	67 ± 13
1–3 months	29	430 ± 119 (272–762)	37 ± 10	30 ± 11 (16–67)	30 ± 11	21 ± 13 (6–56)	11 ± 7	481 ± 127 (324–699)	31 ± 9
4–6 months	33	427 ± 186 (206–1125)	37 ± 16	43 ± 17 (10–83)	43 ± 17	28 ± 18 (8–93)	14 ± 9	498 ± 204 (228–1232)	32 ± 13
7–12 months	56	661 ± 219 (279–1533)	58 ± 19	54 ± 23 (22–147)	55 ± 23	37 ± 18 (16–98)	19 ± 9	752 ± 242 (327–1687)	48 ± 15
13–24 months	59	782 ± 209 (258–1393)	66 ± 18	58 ± 23 (14–114)	59 ± 23	50 ± 24 (19–119)	25 ± 12	870 ± 258 (398–1586)	56 ± 16
25–36 months	33	892 ± 183 (419–1274)	77 ± 16	61 ± 19 (28–113)	62 ± 19	71 ± 37 (19–235)	36 ± 19	1024 ± 205 (499–1418)	65 ± 14
3–5 years	28	929 ± 228 (569–1597)	80 ± 20	56 ± 18 (22–100)	57 ± 18	93 ± 27 (55–152)	47 ± 14	1078 ± 245 (730–1771)	69 ± 17
6–8 years	18	923 ± 256 (559–1492)	80 ± 22	65 ± 25 (27–118)	66 ± 25	124 ± 45 (54–221)	62 ± 23	1112 ± 293 (640–1725)	71 ± 20
9–11 years	9	1124 ± 235 (779–1456)	97 ± 20.	79 ± 33 (35–132)	80 ± 33	131 ± 60 (12–208)	66 ± 30	1334 ± 254 (966–1639)	86 ± 17
12–16 years	9	946 ± 124 (726–1085)	82 ± 11	59 ± 20 (35–72)	60 ± 20	148 ± 63 (70–229)	74 ± 32	1153 ± 169 (833–1284)	74 ± 12
Adults	30	1158 ± 305 (569–1919)	100 ± 26	99 ± 27 (47–147)	100 ± 27	200 ± 61 (61–330)	100 ± 31	1457 ± 353 (730–2365)	100 ± 24

*Reproduced, with permission, from Stiehm ER, Fudenberg HH: *Pediatrics* 1966;37:717.
†Mean ± 1 SD.

trophoresis in agarose gels has also been useful in the diagnosis of certain central nervous system diseases with alterations in cerebrospinal fluid proteins (Fig 17–12).

Oligoclonal bands in cerebrospinal fluid with restricted electrophoretic mobility have been detected in about 90% of clinically definite cases of multiple sclerosis. Agarose electrophoresis gel in conjunction with measurement of cerebrospinal fluid IgG/albumin ratios makes possible a fairly high degree of specificity for diagnosis of multiple sclerosis (see Chapter 32).

Abnormalities in serum proteins other than immunoglobulins may also be detected by serum protein electrophoresis. Hypoproteinemia involving all serum fractions occurs during excessive protein loss, usually in the gastrointestinal tract. Reduction in albumin alone is a common abnormality which occurs in many diseases of the liver, kidneys, or gastrointestinal tract or with severe burns. Alpha$_1$-globulin decrease may indicate α_1-antitrypsin deficiency, and an increase reflects acute phase reactions occurring in many inflammatory and neoplastic disorders. Increase in α_2-globulins usually reflects the nephrotic syndrome or hemolysis with increased hemoglobin-haptoglobin in the serum. Because of its relative insensitivity, zone electrophoresis is almost always a presumptive screening test for serum protein abnormalities. Specific quantitative biochemical or immunologic tests must be performed to definitively identify the protein in question.

IMMUNOELECTROPHORESIS (IEP)

Immunoelectrophoresis combines electrophoretic separation diffusion and immune precipitation of proteins. Both identification and approximate quantitation can thereby be accomplished for individual proteins present in serum, urine, or other biologic fluid.

In this technique (Fig 17–13), a glass slide is covered with molten agar or agarose in a buffer solution (pH 8.2, ionic strength 0.025). An antigen well and antibody trough are cut with a template cutting device. The serum sample (antigen) is placed in the antigen well and is separated in an electrical field with a potential difference of 3.3 V/cm for 30–60 minutes. Antiserum is then placed in the trough, and both serum and antibodies are allowed to diffuse for 18–24 hours. The resulting precipitation lines may then be photographed or the slide washed, dried, and stained for a permanent record.

A comparison of the relationship of precipitation lines developed in normal serum by immunoelectrophoresis and zone electrophoresis is shown in Fig 17–14.

Applications of Immunoelectrophoresis

In the laboratory diagnosis of paraproteinemias, the results of zone electrophoresis and immunoelectrophoresis should be combined. The presence of a

Table 17–2. Serum immunoglobulin levels in disease.*

Diseases	IgG	IgA	IgM
Immunodeficiency disorders			
Combined immunodeficiency	↓↓ ⟷ ↓↓↓	↓↓ ⟷ ↓↓↓	↓↓ ⟷ ↓↓↓
X-linked hypogammaglobulinemia	↓↓ ⟷ ↓↓↓	↓↓ ⟷ ↓↓↓	↓↓ ⟷ ↓↓↓
Common variable immunodeficiency	↓ ⟷ ↓↓↓	↓ ⟷ ↓↓↓	↓ ⟷ ↓↓↓
Selective IgA deficiency	N	↓↓↓	N
Protein-losing gastroenteropathies	N ⟷ ↓↓↓	N ⟷ ↓↓↓	N ⟷ ↓↓↓
Acute thermal burns	N ⟷ ↓↓↓	N ⟷ ↓↓↓	N ⟷ ↓↓↓
Nephrotic syndrome	N ⟷ ↓↓↓	N ⟷ ↓↓↓	N ⟷ ↓↓↓
Monoclonal gammopathies (MG)			
IgG (eg, G-myeloma)	N ⟷ ↑↑↑	N ⟷ ↓↓↓	N ⟷ ↓↓↓
IgA (eg, A-myeloma)	N ⟷ ↓↓↓	N ⟷ ↑↑↑	N ⟷ ↓↓↓
IgM (eg, M-macroglobulinemia)	N ⟷ ↓↓↓	N ⟷ ↓↓↓	N ⟷ ↑↑↑
L chain disease (ie, Bence Jones myeloma)	N ⟷ ↓↓↓	N ⟷ ↓↓↓	N ⟷ ↓↓↓
Chronic lymphocytic leukemia	N ⟷ ↓↓↓	N ⟷ ↓↓↓	N ⟷ ↓↓↓
Infections			
Infectious mononucleosis	↑ ⟷ ↑↑	N ⟷ ↑	↑ ⟷ ↑↑
Subacute bacterial endocarditis	↑ ⟷ ↑↑	↓ ⟷ N	↑ ⟷ ↑↑
Tuberculosis	↑ ⟷ ↑↑	N ⟷ ↑↑↑	↓ ⟷ N
Actinomycosis	↑↑↑	↑↑	↑↑↑
Deep fungus diseases	N	N ⟷ ↑	N
Bartonellosis	↑	↓ ⟷ N	↑↑ ⟷ ↑↑↑
Liver diseases			
Infectious hepatitis	↑ ⟷ ↑↑	N ⟷ ↑	N ⟷ ↑↑
Laennec's cirrhosis	↑ ⟷ ↑↑↑	↑ ⟷ ↑↑↑	N ⟷ ↑↑
Biliary cirrhosis	N	N	↑ ⟷ ↑↑
Chronic active hepatitis	↑↑↑	↑	N ⟷ ↑↑
Collagen disorders			
Lupus erythematosus	↑ ⟷ ↑↑	N ⟷ ↑	N ⟷ ↑↑
Rheumatoid arthritis	N ⟷ ↑↑↑	↑ ⟷ ↑↑↑	N ⟷ ↑↑
Sjögren's syndrome	N ⟷ ↑	N ⟷ ↑	N ⟷ ↑↑
Scleroderma	N ⟷ ↑	N	N ⟷ ↑
Miscellaneous			
AIDS	↑↑	↑↑	↑↑
Sarcoidosis	N ⟷ ↑↑	N ⟷ ↑↑	N ⟷ ↑
Hodgkin's disease	↓ ⟷ ↑↑	↓ ⟷ ↑	↓ ⟷ ↑↑
Monocytic leukemia	N ⟷ ↑	N ⟷ ↑	N ⟷ ↑↑
Cystic fibrosis	↑ ⟷ ↑↑	↑ ⟷ ↑↑	N ⟷ ↑↑

N = normal, ↑ = slight increase, ↑↑ = moderate increase, ↑↑↑ = marked increase, ↓ = slight decrease, ↓↓ = moderate decrease, ↓↓↓ = marked decrease, ⟷ = range.

*Modified and reproduced, with permission, from Ritzmann SE, Daniels JC (editors): *Serum Protein Abnormalities: Diagnostic and Clinical Aspects.* Little, Brown, 1975.

sharp increase or spike in the γ region on zone electrophoresis strongly suggests the presence of a monoclonal paraprotein. However, it is necessary to perform immunoelectrophoresis to determine the exact H chain class and L chain type of the paraprotein. Several examples of the use of immunoelectrophoresis in demonstrating the identity of human serum paraproteins are shown in Fig 17–15.

Immunoelectrophoresis can aid in distinguishing polyclonal from monoclonal increases in γ-globulin (Fig 17–15). Additionally, decreased or absent immunoglobulins observed in various immune deficiency disorders can be analyzed with this technique. However, a further quantitative analysis such as single radial diffusion, nephelometry, or radioimmunoassay should be performed for measurement of immunoglobulin levels.

Immunoelectrophoresis is also of great practical benefit in identifying L chains in the urine of patients with plasma cell dyscrasias or autoimmune disorders. Thus, with specific anti-κ and anti-λ antisera, the monoclonal nature of Bence Jones protein in myeloma can be confirmed.

Antisera to "free light chains" (kappa or lambda) obtained from urine of myeloma patients may occasionally reveal antigenic determinants not present on light chains "bound" to heavy chains. In H chain diseases, fragments of the immunoglobulin H chain are present in increased amounts in the serum (see Chapter 22). It was by careful analysis of immunoelectrophoretic patterns that Franklin initially discovered the existence of this rare but extremely interesting group of disorders. Finally, immunoelectrophoresis is helpful in identifying increased amounts of proteins present in the cerebrospinal fluid in patients with various neurologic diseases.

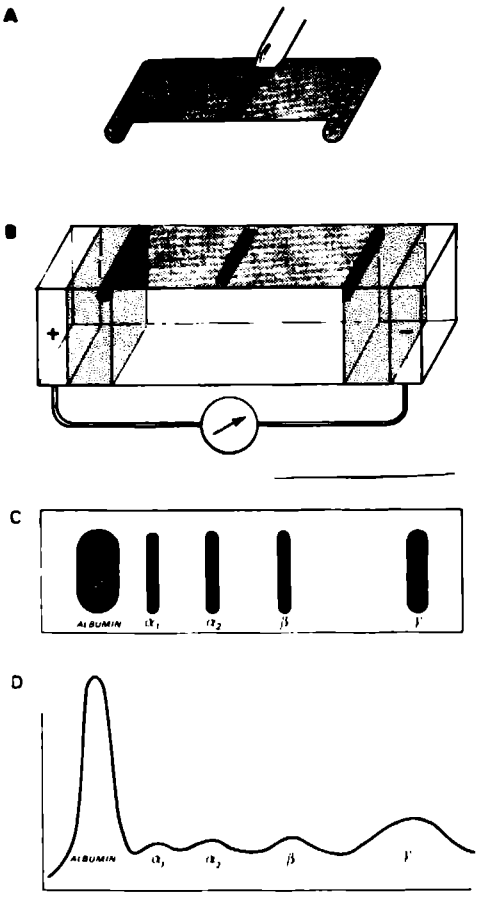


Figure 17–8. Technique of cellulose acetate zone electrophoresis. *A:* Small amount of serum or other fluid is applied to cellulose acetate strip. *B:* Electrophoresis of sample in electrolyte buffer is performed. *C:* Separated protein bands are visualized in characteristic position after being stained. *D:* Densitometer scanning from cellulose acetate strip converts bands to characteristic peaks of albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin.

Immunofixation Electrophoresis

This technique involves separation of proteins electrophoretically in a gel, followed by immunoprecipitation in situ with monospecific antisera. Nonprecipitated proteins are removed by washing and the immunoprecipitation bands revealed with a protein stain. This method has been employed clinically to identify C3 conversion products and to identify paraproteins. The latter is especially helpful for low-level IgM or IgA components, which may be buried in an excess of normal IgG. There are several modifications of this basic method, such as overlay with radioactive or enzyme-linked antibodies that markedly increase the method's sensitivity. In clinical laboratories, its main use is for resolution of serum proteins in difficult diagnostic problems in which results of routine methods are equivocal.

ELECTROIMMUNODIFFUSION

In immunodiffusion techniques described earlier in this chapter, antigen and antibody are allowed to come into contact and to precipitate in agar purely by diffusion. However, the chance of antigen and antibody meeting—and thus the speed of development of a precipitin line—can be greatly enhanced by electrically driving the 2 together. The technique of electroimmunodiffusion is useful in the serologic diagnosis of infectious diseases by serum **antigen** detection. Although numerous variations have been described coupling electrophoresis with diffusion, only 2 have as yet achieved any degree of clinical applicability. These are **one-dimensional double electroimmunodiffusion** (counterimmunoelectrophoresis) and **one-dimensional single electroimmunodiffusion** (Laurell's rocket electrophoresis).

One-Dimensional Double Electroimmunodiffusion

This method is also known as countercurrent immunoelectrophoresis, counterimmunoelectrophoresis, and electroprecipitation. The basic principle of the method involves electrophoresis in a gel medium of antigen and antibody in opposite directions simultaneously from separate wells, with resultant precipitation at a point intermediate between their origins (Fig 17–16).

The principal disadvantages of double diffusion without electromotive force are the time required for precipitation (24 hours) and the relative lack of sensitivity. Double electroimmunodiffusion in one dimension can produce visible precipitin lines within 30 minutes and is approximately 10 times more sensitive than standard double diffusion techniques. However, this technique is only semiquantitative. Some of the antigens and antibodies detected by double electroimmunodiffusion are listed in Table 17–3.

One-Dimensional Single Electroimmunodiffusion

This method is also known as "rocket electrophoresis" or the Laurell technique. The principal application of this technique has been to quantitate antigens other than immunoglobulins. In this technique, antiserum to the particular antigen or antigens one wishes to quantitate is incorporated into an agarose supporting medium on a glass slide in a fixed position so antibody does not migrate. The specimen containing an unknown quantity of the antigen is placed in a small well. Electrophoresis of the antigen into the antibody-containing agarose is then performed. The resultant pattern of immunoprecipitation resembles a spike or rocket—thus the term rocket electrophoresis (Fig 17–17).

This pattern occurs because precipitation occurs along the lateral margins of the moving boundary of antigen as the antigen is driven into the agar containing the antibody. Gradually, as antigen is lost through precipitation, its concentration at the leading edge dimin-

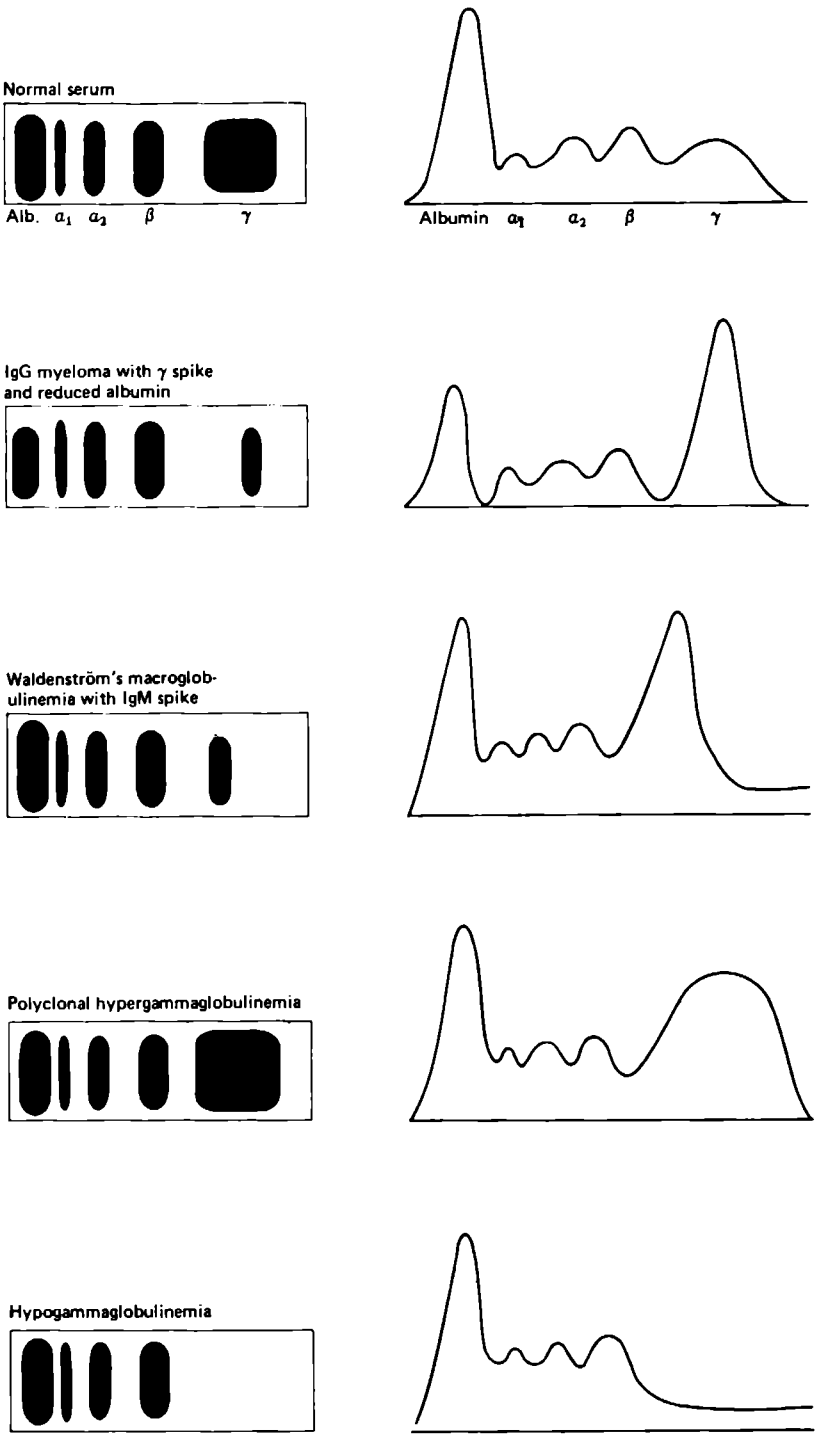


Figure 17–10. Zone electrophoresis patterns of serum immunoglobulin abnormalities in various diseases.

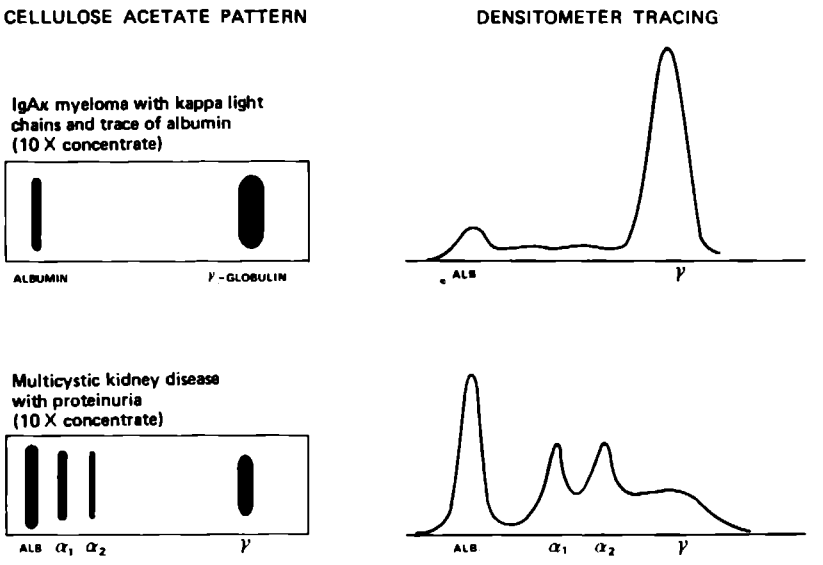


Figure 17–11. Zone electrophoresis patterns of urine abnormalities in various diseases.

ishes and the lateral margins converge to form a sharp point. The total distance of antigen migration for a given antiserum concentration is linearly proportionate to the antigen concentration. The sensitivity of this technique is approximately 0.5 μg/mL for proteins. Unfortunately, the weak negative charge of immunoglobulins prevents their electrophoretic mobility in this system unless special electrolytes and agar are employed. Several commercial systems are available for quantitating serum immunoglobulins and complement components with this technique.

IMMUNOCHEMICAL & PHYSICOCHEMICAL METHODS

Evaluation of serum protein disorders can usually be effectively accomplished by immunodiffusion and electrophoretic methods. Occasionally, more detailed study of immunologically relevant serum constituents

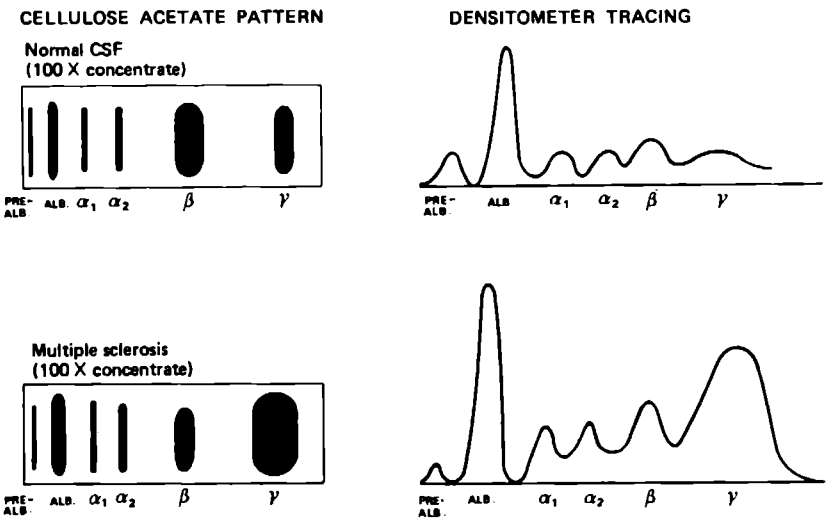


Figure 17–12. Zone electrophoresis patterns of cerebrospinal fluid from normal subject and multiple sclerosis patient.

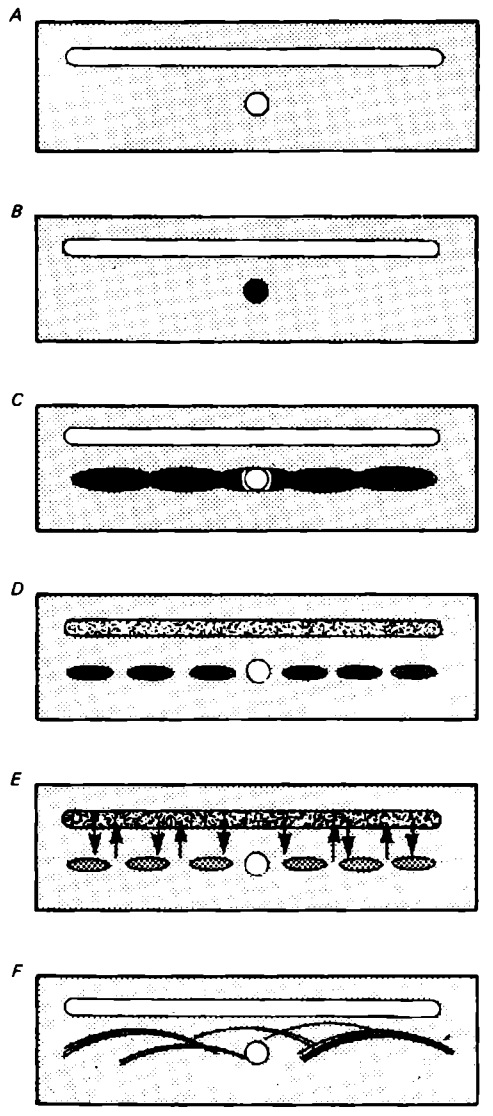


Figure 17–13. Technique of immunoelectrophoresis. *A:* Semisolid agar poured onto glass slide and antigen well and antiserum trough cut out of agar. *B:* Antigen well filled with human serum. *C:* Serum separated by electrophoresis. *D:* Antiserum trough filled with antiserum to whole human serum. *E:* Serum and antiserum diffuse into agar. *F:* Precipitin lines form for individual serum proteins.

is necessary. In this section, a number of the more complex immunochemical and physicochemical techniques are described that have proved to be important adjuncts in the characterization of serum protein disorders. These techniques may be available in the clinical laboratory and include column chromatography, measurement of serum viscosity, and methods to detect cryoglobulins, pyroglobulins, and immune complexes.

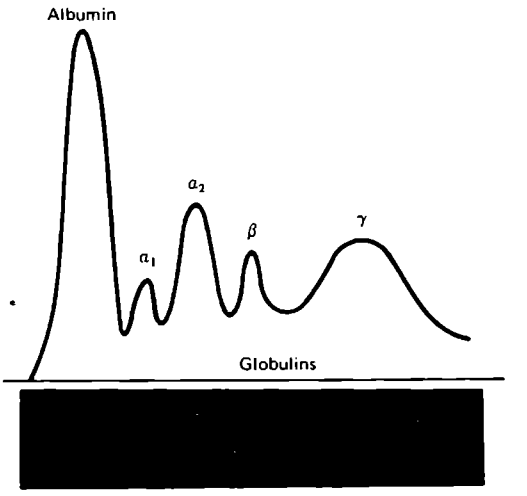


Figure 17–14. Comparison of patterns of zone electrophoresis and immunoelectrophoresis of normal human serum.

COLUMN CHROMATOGRAPHY

Chromatographic techniques are currently the most widely used methods for protein fractionation and isolation of immunoglobulins. In these techniques, a sample is layered on the top of a glass cylinder or column filled with a synthetic gel and is allowed to flow through the gel. The physical characteristics of protein molecules result in retention in the gel matrix to differing degrees, and subsequent elution under appropriate conditions permits protein separation.

Ion Exchange Chromatography

Ion exchange chromatography separates proteins by taking advantage of differences in their electrical charges. The functional unit of the gel is a charged group absorbed on an insoluble backbone such as cellulose, cross-linked dextran, agarose, or acrylic copolymers. Diethylaminoethyl (DEAE), a positively charged group, is the functional unit of anion exchangers used for fractionation of negatively charged molecules (Fig 17–18). Carboxymethyl (CM), negatively charged, is the functional unit of cation exchangers used for fractionation of positively charged molecules. Changing the pH of the buffer passing through the column affects the charge of the protein molecule. Increasing the molarity of the buffer provides more ions to compete with the protein for binding to the gel. By gradually increasing the molarity or decreasing the pH of the elution buffer, the proteins are eluted in order of increasing number of charged groups bound to the gel. For example, Table 17–4 gives the molarity and pH required to elute serum immunoglobulins. DEAE-cellulose chromatography is an excellent technique for isolation of IgG, which can be obtained nearly free of all other serum proteins.

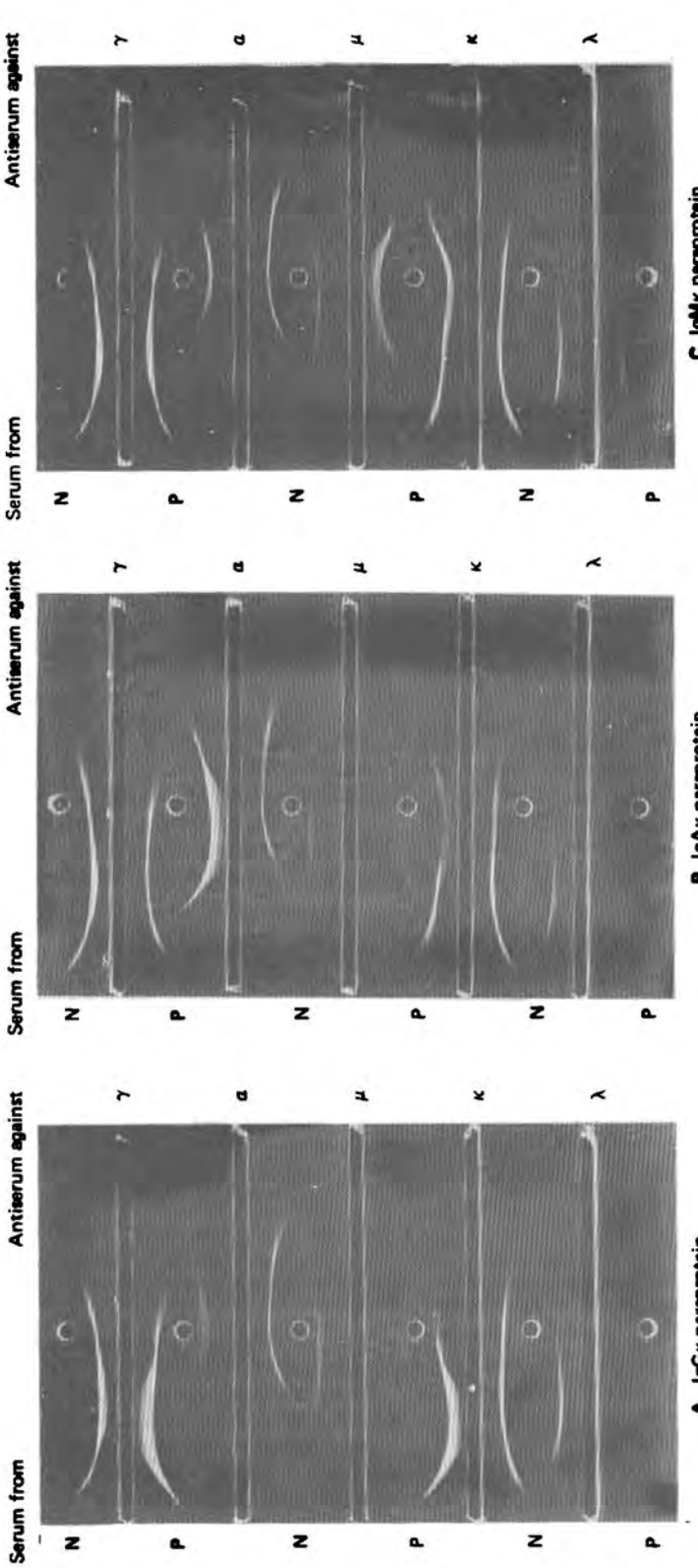
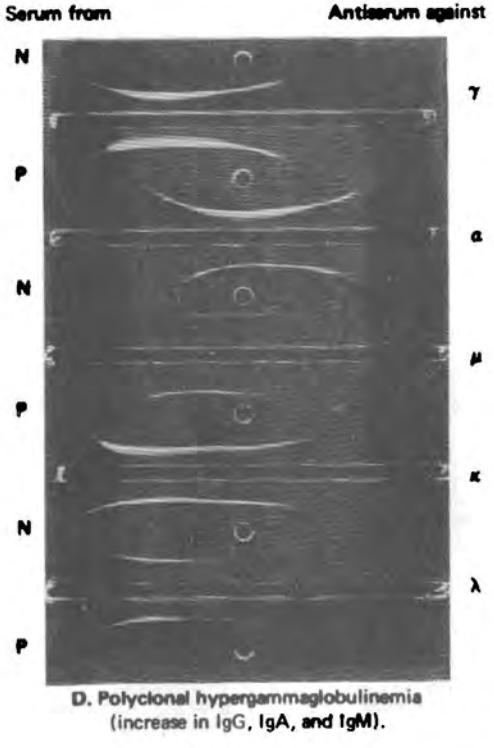


Figure 17–15. Immunoelectrophoresis patterns of serum in various diseases. *A*: IgGκ paraprotein, *B*: IgAκ paraprotein. *C*: IgMκ paraprotein. *D*: Polyclonal hypergammaglobulinemia *E*: Panhypogammaglobulinemia. Individual patterns of serum from normal individual (N) and patient with various serum protein abnormalities (P). In each case, N and P sera are reacted against antisera which are monospecific for γ, α, and μ heavy chains and κ and λ light chains. *D* and *E* are on following page.



D. Polyclonal hypergammaglobulinemia (increase in IgG, IgA, and IgM).

E. Panhypogammaglobulinemia (decreased IgG, IgA, and IgM).

Figure 17–15 (cont'd). Immunoelectrophoresis patterns of serum in various diseases. *A:* IgGκ paraprotein. *B:* IgAκ paraprotein. *C:* IgMκ paraprotein. *D:* Polyclonal hypergammaglobulinemia. *E:* Panhypogammaglobulinemia. Individual patterns of serum from normal individual (N) and patient with various serum protein abnormalities (P). In each case, N and P sera are reacted against antisera which are monospecific for γ, α, and μ heavy chains and κ and λ light chains. *A, B,* and *C* are on previous page.

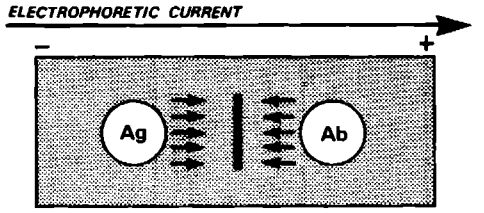


Figure 17–16. Double electroimmunodiffusion in one dimension. Antigen and antibody are placed in well and driven together with an electric current. A precipitin line forms within a few hours after beginning electrophoresis.

Gel Filtration

Gel filtration separates molecules according to their size. The gel is made of porous dextran beads. Protein molecules larger than the largest pores of the beads cannot penetrate the gel pores. Thus, they pass through the gel in the liquid phase outside the beads and are eluted first. Smaller molecules penetrate the beads to a varying extent depending on their size and shape. Solute molecules within the gel beads maintain a concentration equilibrium with solute in the liquid phase outside the beads; thus, a particular molecular species moves as a band through the column. Molecules therefore appear in the column effluent in order of decreasing size (Fig 17–19).

IgM can be easily separated from other serum immunoglobulins by gel filtration. Fig 17–20 shows the separation of the IgM and the IgG components of a mixed IgM-IgG cryoglobulin. Gel filtration is widely used also to separate H and L chains of immunoglobulins or to isolate pure Bence Jones proteins from the urine of patients with multiple myeloma.

Affinity Chromatography

Affinity chromatography uses specific and reversible biologic interaction between the gel material and the substance to be isolated. The specificity of the binding properties is obtained by covalent coupling of an appropriate ligand to an insoluble matrix, such as agarose or dextran beads. The gel so obtained is able to adsorb from a mixed solution the substance to be isolated. After unbound substances have been washed out of the column, the purified compound can be recovered by changing the experimental conditions, such as pH or ionic strength.

Antigen-antibody binding is one of the reactions that can be applied to affinity chromatography. When the gel material is coupled to an antigen, a specific antibody can be purified. Alternatively, when a highly purified antibody can be coupled to the gel, the corresponding antigen can be isolated.

Protein A is a protein isolated from the cell wall of some strains of *Staphylococcus aureus* that specifically reacts with IgG molecules of subclasses 1, 2, and 4. It is used as a specific ligand for isolation of IgG or for isolation of IgG3 from a mixture of IgG molecules of all subclasses.

Cell separation can also be achieved by affinity chromatography. Subpopulations of T and B lymphocytes have been defined by characteristic surface markers (see Chapter 18) that can react with specific ligands. For example, B cells that bear surface immunoglobulins can be separated on an anti-immunoglobulin column. Immunoglobulin-positive cells are retained on the gel, and desorption is achieved by running through the column a solution of immunoglobulins that compete with the cells.

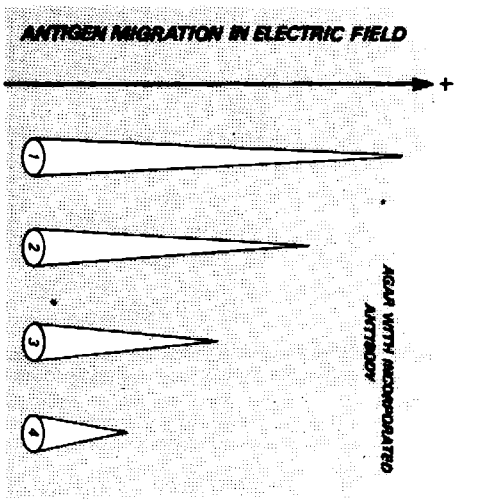


Figure 17–17. Single electroimmunodiffusion in one dimension (rocket electrophoresis, Laurell technique). Antigen is placed in wells numbered 1–4 in progressively decreasing amounts. Electrophoresis is performed and antigen is driven into antibody-containing agar. Precipitin pattern forms in the shape of a "rocket." Amount of antigen is directly proportionate to the length of the rocket.

Table 17–3. Examples of clinical applications of double electroimmunodiffusion.

Cryptococcus-specific antigen in cerebrospinal fluid
Meningococcus-specific antigen in cerebrospinal fluid
Haemophilus-specific antigen in cerebrospinal fluid
Fibrinogen
Cord IgM in intrauterine infection
Carcinoembryonic antigen (CEA)
α_1-Fetoprotein
Fungal precipitins

SERUM VISCOSITY

The measurement of serum viscosity is a simple and valuable tool in evaluation of patients with paraproteinemia. Normally, the formed elements of the blood contribute more significantly to whole blood viscosity than do plasma proteins. However, in diseases with elevated concentrations of serum proteins, particularly the immunoglobulins, the serum viscosity may reach very high levels and result in a characteris-

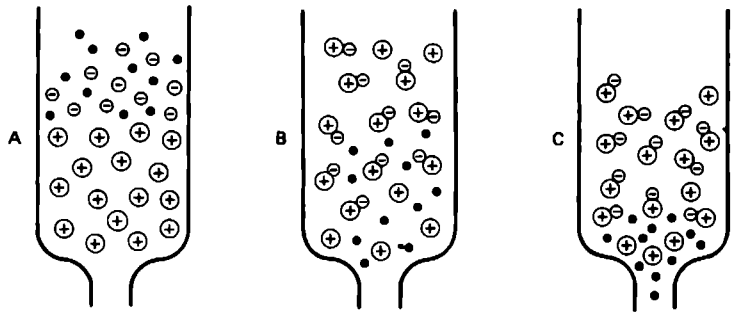


Figure 17–18. Principles of ion exchange chromatography. Three stages of protein separation by ion exchange chromatography are shown: *A:* The column bed is made up of a matrix of positively charged cellulose beads ⊕. *B:* The negatively charged molecules ⊖ in the protein mixture bind to the column and are retained. *C:* The neutral molecules • pass between the charged particles and are eluted.

tic symptom complex—the hyperviscosity syndrome. Serum viscosity is determined by a variety of factors including protein concentration; the size, shape, and deformability of serum molecules; and the hydrostatic state (solvation), molecular charge, and temperature sensitivity of proteins.

In clinical practice, serum viscosity is measured in an Ostwald viscosimeter. A few milliliters of serum are warmed to 37 °C and allowed to descend through a narrow bore capillary tube immersed in a water bath at 37 °C. The rate of descent between calibrated marks on the capillary tube is recorded. The same procedure is repeated using distilled water. The relative serum viscosity is then calculated according to the following formula:

$$\text{Relative serum viscosity} = \frac{\text{Rate of descent of serum sample (in seconds)}}{\text{Rate of descent of distilled water (in seconds)}}$$

Normal values for serum viscosity range from approximately 1.4 to 1.9. Similar measurements can be performed using plasma instead of serum. However, fibrinogen present in plasma is a major determinant of plasma viscosity, and variations in this protein, especially in the presence of nonspecific inflammatory states, can markedly affect the results. For this reason, measurement of serum viscosity is preferred.

Serum viscosity measurements are primarily of use in evaluating patients with Waldenström's macroglobulinemia, multiple myeloma, and cryoglobulinemia. In myeloma, aggregation or polymerization of the paraprotein in vivo often results in hyperviscosity. In general, there is a correlation between increased serum viscosity and increased plasma volume. However, the correlation between levels of relative serum viscosity and clinical symptoms is not nearly as direct. Increased serum viscosity may interfere with various laboratory tests that employ flow-through devices such as Coulter counters and Technicon analyzers in clinical chemistry. A detailed discussion of the hyperviscosity syndrome is presented in Chapter 22. Disorders with increased serum viscosity are listed in Table 17–5.

CRYOGLOBULINS

Precipitation of serum immunoglobulins in the cold was first observed in a patient with multiple myeloma. The term "cryoglobulin" was introduced to designate a group of proteins which had the common property of forming a precipitate or a gel in the cold. This phenomenon was reversible by raising the temperature. Since those initial descriptions, cryoglobulins have been found in a wide variety of clinical situations. Purification and immunochemical analysis have led to classification of this group of proteins (Table 17–6). Type I cryoglobulins consist of a single monoclonal immunoglobulin. Type II cryoglobulins are mixed cryoglobulins; they consist of a monoclonal im-

Table 17–4. Molarity of NaCl and pH required to elute human plasma protein from DEA-cellulose. (Adapted from Oh and Sanders, 1966.)

NaCl Molarity	pH	Proteins Eluted
0.025	7.8	IgG
0.045	7.0	Transferrin, fibrinogen
0.050	7.0	α_2-Globulin, albumin, IgA
0.080	6.5	Albumin, α_2-globulin, β-lipoprotein
0.100	6.5	α_1-Globulin, β-globulins, haptoglobin
0.150	6.5	IgM, β-lipoprotein, β-globulin

Table 17–5. Disorders with increased serum viscosity.

Waldenström's macroglobulinemia
Essential macroglobulinemia
Multiple myeloma
Cryoglobulinemia
Hypergammaglobulinemic purpura
Rheumatoid diseases associated with immune complexes or paraproteinemias
  Rheumatoid arthritis
  Sjögren's syndrome
  Systemic lupus erythematosus

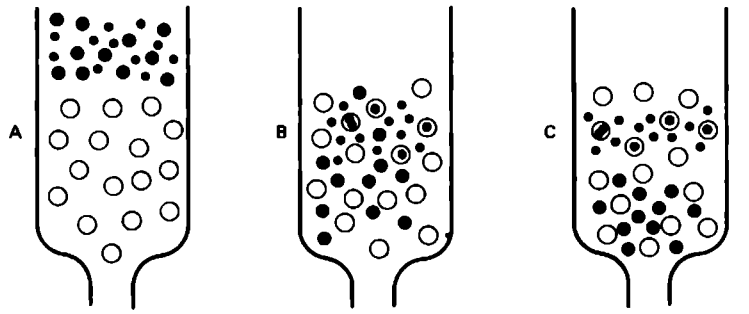


Figure 17–19. Principles of gel filtration chromatography. Three stages of protein separation by gel filtration are shown: *A:* Open circle ○ represents polymerized beads onto which a mixture of small • and large ● protein molecules is layered. *B:* The molecules enter and pass through the column at different rates depending primarily on size and are separated by a simple sieving process. *C:* Larger molecules are eluted while smaller ones are retained.

munoglobulin with antibody activity against a polyclonal immunoglobulin. Type III cryoglobulins are mixed polyclonal cryoglobulins, ie, one or more immunoglobulins are found, none of which are monoclonal.

Technical Procedure for Isolation & Analysis

Blood must be collected in a warm syringe and kept at 37 °C until it clots. Serum is separated by centrifugation at 37 °C and then stored at 4 °C. When a cryoglobulin is present, a white precipitate or a gel appears in the serum after a variable period, usually 24–72 hours. However, the serum should be observed for 1 week to make certain that unusually late cryoprecipitation does not go undetected. The reversibility of the cryoprecipitation should be tested by rewarming an aliquot of precipitated serum.

Quantitation of the cryoprecipitate can be done in several ways. Centrifugation of the whole serum in a hematocrit tube at 4 °C allows determination of the relative amount of cryoglobulin (cryocrit). Alternatively, the protein concentration of the serum may be compared before and after cryoprecipitation. The precipitate formed in an aliquot of serum may be isolated and dissolved in an acidic buffer and the cryoglobulin level estimated by the absorbance at 280 nm.

After isolation and washing of the precipitate, the components of the cryoglobulin are identified by immunoelectrophoresis or by double diffusion. These analyses are performed at 37 °C, using antiserum to whole human serum and antisera specific for γ, α, μ, κ, and λ chains. In this way, cryoglobulins can be classified into the 3 types described above.

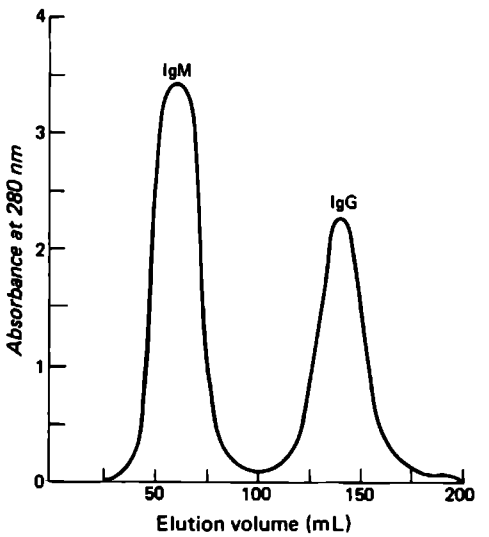


Figure 17–20. Separation of IgG-IgM mixed cryoglobulin by gel filtration. Two peaks are eluted from gel filtration column. The larger IgM molecules precede the smaller IgG molecules which were dissociated by dissolving the cryoprecipitate in an acidic buffer prior to application to the column. The absorbance at 280 nm measures relative amount of protein in various eluted fractions.

Clinical Significance

Type I and type II cryoglobulins are usually present in large amounts in serum (often more than 5 mg/mL). In general, they are present in patients with monoclonal paraproteinemias, eg, they are commonly found in patients with lymphoma or multiple myeloma. However, some are found in patients lacking any evidence of lymphoid malignancy, just as are "benign" paraproteins (see Chapter 22). Type III cryoglobulins indicate the presence of circulating immune complexes and are the result of immune responses to various antigens. They are present in relatively low concentrations (usually less than 1 mg/mL) in rheumatoid diseases and chronic infections (Table 17–6).

All types of cryoglobulins may be responsible for specific symptoms that occur as a result of changes in the cryoglobulin induced by exposure to cold. The symptoms include Raynaud's phenomenon, vascular purpura, bleeding tendencies, cold-induced urticaria, and even distal arterial thrombosis with gangrene.

Table 17–6. Classification of types of cryoglobulins and associated diseases.

Type of Cryoglobulin	Immunochemical Composition	Associated Diseases
Type I		
Monoclonal cryoglobulin	IgM IgG IgA Bence Jones protein	Myeloma, Waldenström's macroglobulinemia, chronic lymphocytic leukemia
Type II		
Mixed cryoglobulin	IgM-IgG IgG-IgG IgA-IgG	Myeloma, Waldenström's macroglobulinemia, chronic lymphocytic leukemia, rheumatoid arthritis, Sjögren's syndrome, mixed essential cryoglobulinemia
Type III		
Mixed polyclonal cryoglobulin	IgM-IgG IgM-IgG-IgA	Systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, infectious mononucleosis, cytomegalovirus infections, acute viral hepatitis, chronic active hepatitis, primary biliary cirrhosis, poststreptococcal glomerulonephritis, infective endocarditis, leprosy, kala-azar, tropical splenomegaly syndrome

Since type II and type III cryoglobulins are circulating soluble immune complexes, they may be associated with a serum sickness-like syndrome characterized by polyarthritis, vasculitis, glomerulonephritis, or neurologic symptoms (see Chapter 21). In patients with mixed essential IgM-IgG cryoglobulinemia, a rather distinctive syndrome may occur that is associated with arthralgias, purpura, weakness, and frequently lymphadenopathy or hepatosplenomegaly. This syndrome may be a sequela of hepatitis B infection. Glomerulonephritis is a common finding. In some instances, it occurs in a rapidly progressive form and is of ominous prognostic significance.

Cryoglobulins may cause serious errors in a variety of laboratory tests by precipitating at ambient temperatures and thereby removing certain substances from serum. Complement fixation and inactivation and entrapment of immunoglobulins in the precipitate are common examples. Redissolving the cryoprecipitate usually does not restore activity to the serum, especially when measuring complement activity.

PYROGLOBULINS

Pyroglobulins are monoclonal immunoglobulins that precipitate irreversibly when heated to 56 °C. This phenomenon is different from the reversible thermoprecipitation of Bence Jones proteins and seems to be related to hydrophobic bonding between immunoglobulin molecules, possibly due to decreased polarity of the heavy chains. Pyroglobulins may be discovered incidentally when serum is heated to 56 °C to inactivate complement before routine serologic tests. Half of them are found in patients with multiple myeloma. The remainder occur in macroglobulinemia and other lymphoproliferative disorders, systemic lupus erythematosus, and carcinoma and occasionally without known associated disease. They are not responsible for any particular symptom except hyperviscosity and have no known significance.

DETECTION OF IMMUNE COMPLEXES

The factors involved in deposition of immune complexes in tissues and production of tissue damage are discussed in Chapter 11. Subsequent chapters deal with the clinical manifestations of diseases associated with immune complexes, including rheumatic diseases (Chapter 21), hematologic diseases (Chapter 22), and renal diseases (Chapter 28). These clinical situations have in common the presence of detectable immune complexes in tissues or in the circulation.

Detection of Immune Complexes in Tissues

Detection of immune complexes in tissues is performed by immunohistologic techniques using immunofluorescence or immunoperoxidase staining. The antisera used are specific for the immunoglobulin classes, complement components, fibrin or fibrinogen, and, in selected cases, the suspected antigen.

By analogy with animal findings, granular deposits of immunoglobulins usually accompanied by complement components are considered to represent immune complexes. The antigen moiety is rarely detected, since it is unknown in most cases.

Detection of Immune Complexes in Serum & Other Biologic Fluids

Until recent years, the presence of circulating immune complexes could only be indirectly inferred when complement levels measured by C3 or CH_{50} were low or when serum was found to be anticomplementary during the performance of a serologic test using complement fixation. The availability of more selective means for detection of circulating immune complexes has become increasingly necessary in clinical immunology. Immune complex determination is used as a diagnostic criterion for various diseases as an estimate of their severity, as an index to monitor the results of treatment in patients with immune complex diseases, and as a research tool in the investigation of the pathogenetic basis of immunologic diseases. A variety of methods has been developed recently in an attempt to achieve maximum sensitivity, specificity, and reproducibility while keeping the technical procedure simple enough to be used as a routine test.

Table 17–7. Methods for detection of circulating immune complexes.

Physical methods
Ultracentrifugation
Gel filtration
Cryoprecipitation
Precipitation with polyethylene glycol
Nephelometry
Interaction with rheumatoid factor or complement
Precipitin reactions
Inhibition of agglutination of IgG-coated latex particles
Anticomplementary activity
Solid phase radioassay
C1q binding test
C1q deviation test
Conglutinin binding test
Interaction with cell receptors
Platelet aggregation test
Inhibition of phagocytosis of labeled aggregates
Inhibition of EAC rosette formation
Raji cell test

Methods currently in use are based on different biologic or chemical properties of immune complexes. As a result, they detect complexes of various sizes and properties, and none of the methods are satisfactory for all types. When possible, detection of circulating immune complexes should be done by several techniques.

A. Physical Methods: Ultracentrifugation and gel filtration can be used, although they are not very sensitive methods and are usually used for separation rather than detection of immune complexes.

Cryoglobulins of types II and III, when detected in biologic fluids, represent immune complexes which can be easily isolated and characterized. However, cryoprecipitation is not a universal property of antigen-antibody complexes.

Owing to their large size, immune complexes can be precipitated by high-molecular-weight polymers such as polyethylene glycol even at low concentrations of complexes which may leave soluble antigen or antibody as a residual in the reaction. Precipitated immune complexes are quantitated by measuring the protein content of the resolubilized precipitate (absorbance at 280 nm) or its concentration of immunoglobulins or complement components (radial diffusion).

B. Interaction With C1q, Rheumatoid Factor, or Conglutinin: Precipitin reactions were first used to detect interaction of immune complexes with C1q or rheumatoid factor. They have been superseded by more sensitive methods.

Rheumatoid factor and C1q are able to agglutinate latex particles coated with aggregated IgG. When mixed with immune complexes, active sites on the latex particles are blocked, thereby preventing their subsequent agglutination by rheumatoid factor or C1q. Inhibition of the latex agglutination test can therefore be used for detection of immune complexes.

Binding of immune complexes to C1q leads to activation of complement system, thereby depressing its hemolytic activity. This is the principle of the measure of anticomplementary activity of immune complexes in serum. The sample to be tested is freed from autologous complement activity by heat inactivation. It is then mixed in various dilutions with fresh normal serum which serves as a source of normal complement activity. Hemolytic activity (CH_{50}) of the fresh normal serum is measured with and without the addition of the sample. The anticomplementary activity is expressed as the percentage of reduction of CH_{50}.

Several techniques use radioisotopes either in solid or liquid phase to measure C1q binding. For **solid phase radioassay,** C1q is adsorbed on plastic polystyrene tubes. The sample is incubated in the coated tube, then washed out. The amount of immune complexes bound to C1q is estimated by binding of radiolabeled anti-immunoglobulin antibody or by binding of radiolabeled aggregated IgG onto free C1q. Alternatively, enzyme-conjugated anti-immunoglobulin reagents can be substituted here in a typical ELISA method. In the **liquid phase assay,** the sample is incubated with soluble radiolabeled C1q. In the C1q binding test, bound C1q is precipitated by polyethylene glycol and radioactivity measured in the precipitate. In the C1q deviation test, remaining free C1q is fixed on sensitized erythrocytes in such conditions that hemolysis does not occur. Radioactivity is measured in the erythrocyte pellet.

Conglutinin, a bovine serum protein, is known to react with fixed C3. This property is used in the **conglutinin binding test.** The sample is incubated with conglutinin, which is adsorbed onto plastic tubes. After washing, conglutinin-bound immune complexes are measured by the uptake of enzyme-conjugated or radiolabeled anti-immunoglobulin antibody.

C. Interaction With Cell Receptors: Antigen-antibody complexes can interact with the membrane of platelets. This interaction produces changes on the platelets which can be revealed by platelet aggregation **(platelet aggregation test).**

Immune complexes can be phagocytized in vitro by macrophages, as can other large molecular complexes. This is used in a **phagocytosis inhibition test.** Peritoneal macrophages are incubated with the sample to be tested and with radiolabeled aggregated IgG. The presence of immune complexes in the sample is revealed by a decrease in the uptake of radioactivity by the cells compared to a control where incubation takes place with aggregates alone.

B cells have surface receptors for the third component of complement (C3) through which they can bind complement-fixing immune complexes. When peripheral blood lymphocytes are used as a source of B cells, fixation of immune complexes to their surface is revealed by a reduction of the number of EAC rosette-forming cells (see Chapter 18). Cultured lymphoblastoid B cell lines can also be used. The cells from the Raji line have receptors for C3 but lack surface immunoglobulins. Immune complexes bound to their surface can thus be estimated by secondary fixation of a radiolabeled anti-immunoglobulin antibody without interference by surface immunoglobulins.

Clinical Usefulness of Immune Complex Determinations

Initial enthusiasm regarding possible clinical benefits of measuring immune complexes has been tempered by their relative lack of diagnostic or prognostic specificity. Circulating complexes can occur in the absence of tissue deposition, and occasionally no serum complexes can be found despite tissue deposition. In addition, the considerable potential for uncovering causes of many idiopathic diseases by isolating and identifying the antigen in immune complexes has not yet been realized. There are many published discrepancies among the results of various assays. Nevertheless, immune complexes have pathogenic roles depending on their size, immunoglobulin class, concentration, and affinity for cellular receptors. Most experts would probably agree that immune complex determinations are in the realm of emerging rather than proved clinical usefulness.

NEPHELOMETRY

Nephelometry is measurement of light that is scattered from the main beam of a transmitted light source. The term is often confused with turbidimetry, which is the measurement of the decrease of light passing through a cloudy solution or suspension of material. In dilute solutions, the precipitation reaction between antigen and antibody produces increased reflection that can be measured by the scattering of an incident light source. Devices to measure light scattering produced by reaction of diphtheria toxin and antitoxin were introduced by Libby in 1938, and this technique has recently received increasing application in the clinical laboratory.

Nephelometric determination of antigens is performed by addition of constant amounts of highly purified and optically clear specific antiserum to varying amounts of antigen. The resultant antigen-antibody reactants are placed in a cuvette in a light beam of various wavelengths and the degree of light scatter measured in a photoelectric cell as the optical density (Fig 17–21). Accurate measurement of antigens can only be made in the ascending limb of the precipitin curve (Fig 17–1), since at equivalence and in antibody excess there is no direct linear relationship between antigen concentration and optical density. Thus, for accurate determination of solutions with high antigen concentrations, the samples must be diluted.

There are several different approaches to applying nephelometry in the clinical laboratory. Automated immunoprecipitation employs a fluorometric nephelometer in line with a series of flow-through channels that allow for the measurement of multiple samples simultaneously. Laser nephelometers employ a helium-neon laser beam as a light source and sensitive detection devices to measure forward light scatter. Introduction of various electronic filters near the detection device assures a high "signal-to-noise" ratio and a relatively high degree of sensitivity. A modified centrifugal fast analyzer equipped with a laser light source

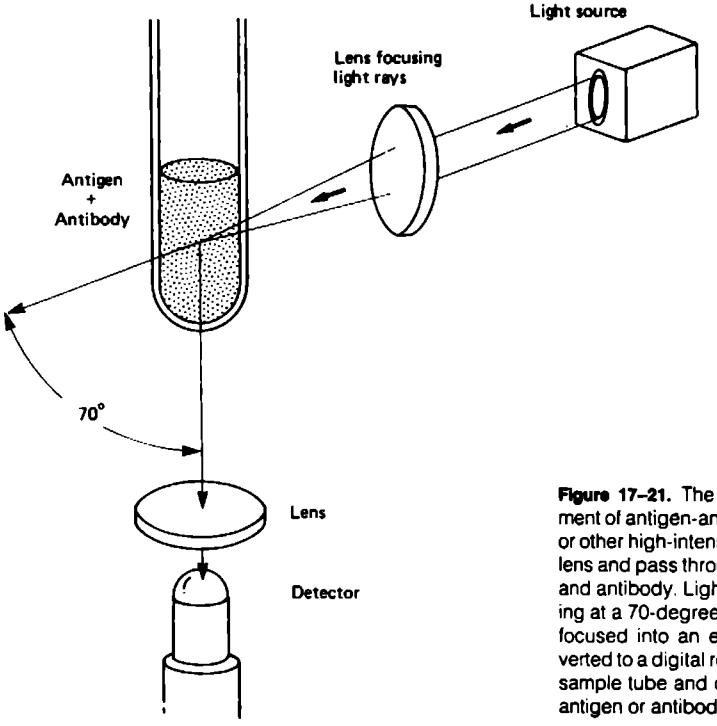


Figure 17–21. The principle of nephelometry for measurement of antigen-antibody reactions. Light rays from a laser or other high-intensity source are collected in the focusing lens and pass through the sample tube containing antigen and antibody. Light passing through the tube and emerging at a 70-degree angle is collected by another lens and focused into an electronic detector. This signal is converted to a digital recording of the amount of turbidity in the sample tube and can be mathematically related to either antigen or antibody concentration in the sample.

has also been employed for scatter measurements. This method has the potential advantages of speed, low amounts of reagents required, and versatility for other assays.

Nephelometry is theoretically a rapid and simple method for quantitation of many antigens in biologic fluids. Disadvantages of the technique include relatively high cost of optically clear, potent antisera of uniform specifications; high background resulting from sera containing lipids or hemoglobin; and the need for multiple dilutions, especially for high antigen concentrations. However, some of these potential sources of error are inherent in other immunoquantitative methods. Many of these inherent disadvantages can be overcome by the use of **rate nephelometry.** In this technique, a nephelometer electronically subtracts background signals. More precise measurement of turbidity is achieved by taking several measurements rapidly during the ascending phase of the precipitation reaction. Nephelometers that combine many of these features are now commercially available. The widespread use of such instruments—and nephelometric grade reagents—has made this method cheaper and applicable to many immunochemical determinations.

BINDER-LIGAND ASSAY

Unquestionably one of the most important analytic methods developed in the past quarter century is binder-ligand assay (also known in various periods of its development as **ligand assay, competitive protein binding assay,** and **saturation analysis**). The first ligand assay method was **radioimmunoassay (RIA),** the first of which was developed to detect human insulin, utilizing human anti-insulin antibodies. It was described in papers appearing in 1959–1960 by Berson and Yalow. Their discovery that the body manufactures antibodies against endogenous substances went against a fundamental dictum of the time which held that the body could not make antibodies against itself. This discovery was in certain respects as important as the application of these antibodies to a new assay method. Yalow shared the Nobel Prize for Physiology or Medicine in 1977 for her contributions.

Simultaneously and independently, Ekins was developing an assay for human thyroid hormone using thyroid-binding globulin isolated from a patient with elevated levels of this binding protein. The basic principle of his assay was the same, although it used a serum carrier protein rather than an antibody. In the 2 decades since its inception, ligand assay has revolutionized disciplines within biology and medicine. It has been applied to quantitation of hormones, drugs, tumor markers, and antibodies associated with allergy. The rapid detection of bacterial and viral infections has been made possible, as well as the detection of antibodies associated with infectious diseases such as hepatitis and AIDS. With modern antibody production methods, monoclonal and polyclonal antibodies can be produced against many substances.

RADIOIMMUNOASSAY (RIA)

The chief goal of an assay is to determine the concentration of some molecule of interest, the **analyte.** Common to all of the ligand assay methods is the reaction of analyte with a binding protein, or **binder,** which most often is an **antibody.** In its role as a reactant with binder, the analyte is referred to as a **ligand.** Because the analyte may be smaller than molecules normally capable of stimulating antibody production, it may at some point have been attached to a larger molecule to enable antibody production; in this role, the analyte/ligand is known as a **hapten.** In certain assay designs, binder may be reacting with multiple distinct ligands, including the analyte.

There is a bewildering array of ligand assay methods; only the first member of the ligand assay family, radioimmunoassay (RIA), will be described in full, followed by (in very broad terms) the chemical principles underlying some of the more important alternative ligand assay methods. Any ligand assay can be divided into 3 stages: calibration, interpolation, and quality control.

Calibration

The binder-ligand reaction. A number of reaction vessels are established, each containing a small fixed concentration of binder and a small fixed concentration of radioisotopically labeled analyte known as the **label** or **tracer** (Fig 17–22). Calibration requires a set of dilutions of analyte of known concentration; these are referred to as the **standards** or **calibrators.** Different known amounts of calibrator are added to a series of antibody/label mixtures. The central event in RIA is the competition between the label and the (unlabeled) analyte for binding sites on an antibody. According to the degree of completion of the binder-ligand chemi-

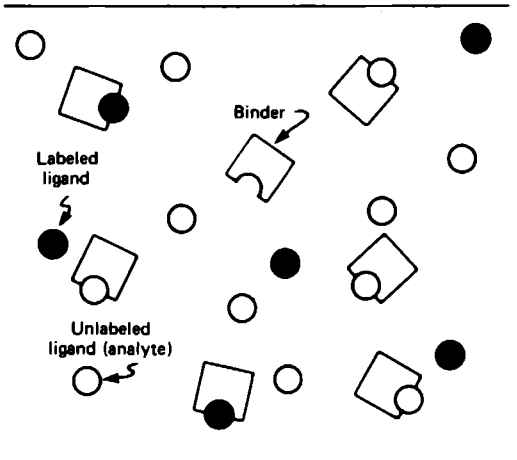


Figure 17–22. The binder-ligand reaction underlying RIA.

cal reaction, the assay is said to be either an **equilibrium assay** (the reaction is complete) or a **disequilibrium assay** (the reaction is incomplete). The label is divided into 2 categories by the reaction: label which is bound to antibody (the **bound fraction**) and label which is free in solution (the **free fraction**). As the amount of analyte increases relative to the small fixed amount of label present, an increasing fraction of the label will be free.

Partitioning and separation. The bound and free fractions are subjected to a **partitioning step,** after which they are physically separated. Partitioning methods that sequester the binder and binder-ligand complexes include **salting out** of protein (using ammonium or sodium sulfate), **protein denaturation/precipitation** by solvent (such as methanol, ethanol, or acetone), and **precipitation** by polyethylene glycol or by a **second antibody** directed against the primary antibody. Immobilization of the binder to a **solid phase** (the assay reaction tube or a macroscopic particle) has been successful, although it requires care in manufacture to ensure reproducible behavior. A common method of acting upon the free fraction is **adsorption of free ligand** (using talc, charcoal, silica, ion exchange resin, cellulose, Sephadex, or fuller's earth).

Other methods for partitioning that are rarely used include **electrophoresis, gel filtration,** and **equilibrium dialysis.**

Following partitioning, the bound and free fractions are subjected to **physical separation.** During this step, some mixing of the 2 fractions occurs, leading to misclassification of the fractions.

Measurement of response. In radioassays, the final measurement method is **radioactive counting,** the method for which depends upon the type of radiation emitted by the label. A **liquid scintillation counter** is used for alpha or beta emitters and a **solid crystal gamma counter** for gamma emitters. The final measurement, or some computed value derived from it, is known as the **response.** The choice of an appropriate response is dictated by the statistical requirements of data reduction. A commonly employed value in RIA is the ratio of bound to total label, or B:T.

Creation of a calibration curve. The physical and chemical steps taken thus far are referred to as the **analytic method** of the assay. We now leave the realm of chemistry and enter the realm of mathematics, for a relationship between calibrator concentration and assay response can now be established. Most currently encountered assay calibration curves are roughly symmetric sigmoid curves when plotted using a logarithmic concentration axis (Fig 17–23).

This sort of curve can usually be characterized by the 4-parameter logistic equation

$$y = \left[\frac{a-d}{a+(x/c)^b}\right] + d \qquad \ldots(1)$$

where a is the upper asymptote, d is the lower asymptote, c is the concentration corresponding to the response $(a+d)/2$, and b is related to the slope at this point. Earlier workers used a simplified form of this equation, the **logit transformation,** which lends itself well to manual plotting. If one defines a new response value as $y' = (y-d)/(a-d)$, the logit transformation proceeds as follows:

$$Y = \text{logit}(y') = \ln\left[\frac{y'}{1-y'}\right] \qquad \ldots(2)$$

Note the similarity of this approach to that of the von Krogh equation discussed later in this chapter. If the data really follow a symmetric sigmoid on the log-linear plot, then a plot of Y versus log concentration will be a straight line. The term b in the 4-parameter logistic equation is simply the slope of the line in the logit-log coordinate system.

Interpolation of Test Concentrations

Once a calibration relationship is in hand, we are ready to estimate the concentration of analyte in **test specimen "unknowns."** These specimens are processed just as the calibrators were, and a response is obtained. This response is used in conjunction with the calibration curve to find a **concentration estimate** (or dose estimate) corresponding to the observed response; this process is known as **interpolation** (or dose interpolation).

Error Computations & Quality Control

The goal in obtaining an analyte concentration estimate is generally to answer a question such as "Is the analyte concentration larger (or smaller) than a given dangerous (or therapeutic) level?" or "Is the concentration larger (or smaller) than some previously measured concentration?" Because random errors are involved in any measurement, an assay result should be accompanied by statistically determined **confidence limits** to aid in such judgments. Such confidence limits define a zone within which the actual results would

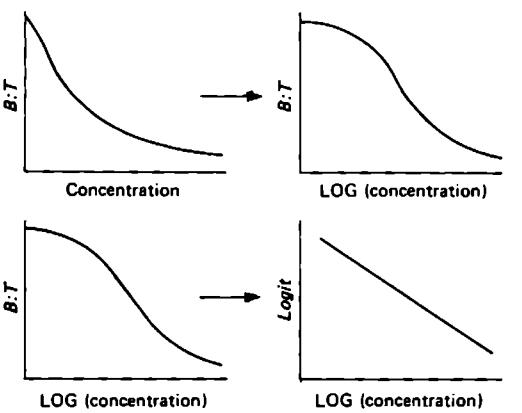


Figure 17–23. The logistic equation and the logit transformation.

be expected to fall at some stated level of probability. Furthermore, it is desirable to control the level of both random and systematic error in assay results, and so certain quality control procedures should be followed with each assay batch to allow the rejection of results likely to contain extraordinarily large error. There are many varieties of quality control tests: one of them relies upon analysis of quality control specimens of known concentration in each assay batch.

VARIATIONS IN ANALYTIC METHOD

Radioisotopic Labels

There has been an explosion of methods based on various modifications of this initial assay scheme, arbitrarily divided according to their labeling methods.

Immunoradiometric assay (IRMA). In this method, the binder (generally an antibody) is labeled rather than the ligand.

Sandwich assay. There are numerous variations of this technique. In its most basic form, ligand reacts with an antibody that has been immobilized upon a solid surface. Then a radiolabeled second antibody is added, which reacts with ligand at a different site. This method has the potential advantage of added chemical specificity owing to the use of 2 distinct antigenic sites (Fig 17–24).

Enzymatic Labels

Enzyme-multiplied immunoassay (EMIT). The name of this method is misleading and has led some to think that precision or sensitivity is enhanced, which is not true. The label consists of ligand conjugated to an enzyme, which remains active (Fig 17–25). Binding of antibody to the enzyme-ligand complex inactivates the enzyme; the presence of free ligand (analyte) competes with the enzyme-ligand complex for antibody, increasing the resulting enzymatic activity. Over a limited range, the enzyme activity will be approximately proportionate to analyte concentration. This method has been widely employed for therapeutic drug monitoring.

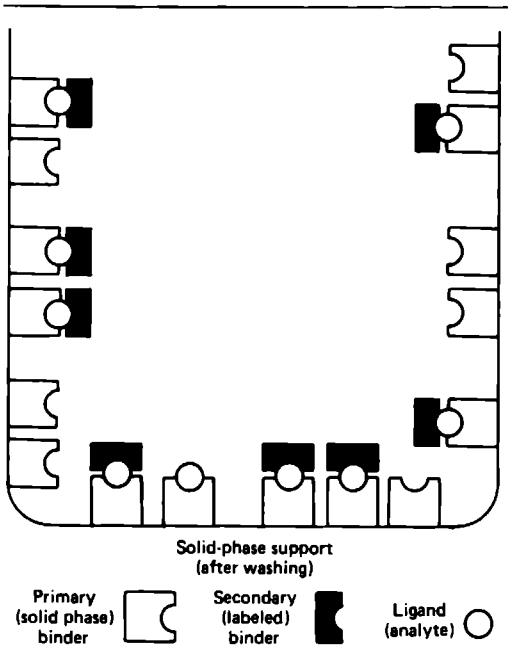


Figure 17–24. Sandwich assay.

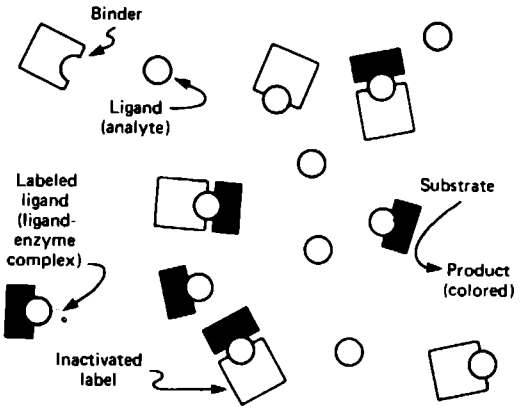


Figure 17–25. EMIT.

Enzyme-linked immunoabsorbent assay (ELISA). This is nothing more than an enzymatic variation on the sandwich assay method; the twist is that one is attempting to detect an antibody, so that the roles of binder and ligand are reversed (Fig 17–26). The solid-phase component is an antigen. The antibody to be detected binds to this component, and then a second (enzyme-labeled) antibody directed against the antibody to be detected is added. This test is frequently used by clinical immunologists.

Fluorometric Labels

Pulsed-light time-resolved fluorometric immunoassay. Fluorometric assays pose several unique problems. Serum has substantial fluorescence of its own, and the scattering produced by its larger constituents interferes with the performance of fluorescent labels. Chelates of rare earth metals (dysprosium, europium, samarium, terbium) possess a long decay time in their fluorescence. Time-resolved fluorometers produce a fast excitation pulse and then delay for a short interval of time (on the order of nanoseconds) before measuring fluorescence. Background fluorescence dies away during the delay, but the rare-earth label continues to fluoresce.

Fluorescence-polarization immunoassay (FPIA). The label is coupled by means of the analyte to a fluorescein derivative (Fig 17–27). When free in solution, tracer molecules tumble randomly and so rapidly that when excited by polarized light, emitted light is

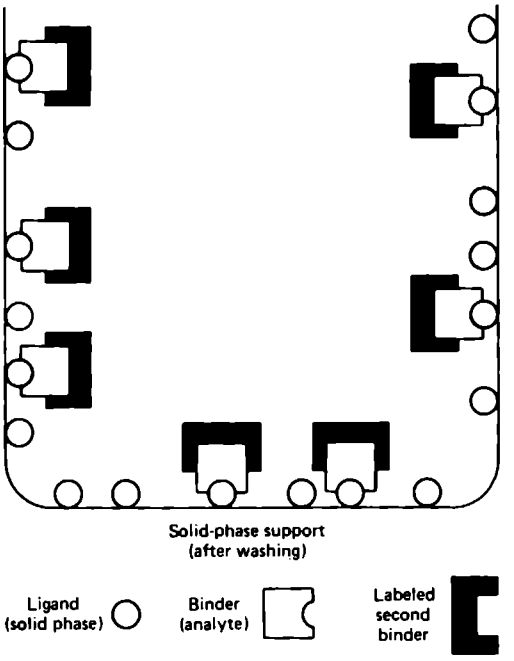


Figure 17–26. ELISA.

unpolarized. When the tracer is bound to an antibody, the tumbling is slowed and the emitted light is more polarized. The degree of polarization will reflect the amount of label that is bound. Thus far, only small analytes have been measurable by this means.

Particle concentration fluorescence immunoassay (PCFIA). This is a sandwich assay that can take several forms (Fig 17–28). In one form, the first binder is immobilized upon microscopic latex spheres. A second, fluorescently tagged binder reacts with the binder-ligand complex, and after washing, the latex spheres are concentrated onto the bottom of the reaction well where their fluorescence is determined. If the substance immobilized to the spheres is a hapten, this method can be used to quantitate antibody.

Other Variations

Other methods that have been studied include systems employing liposomes or red blood cells, methods using nephelometry, assays using metal atoms as labels (detected by atomic absorption), and assays using electron-spin resonance ("spin labeling"). Enzymatic and nonenzymatic methods have been pursued to yield assays that use electrochemical detection methods. Enzymes from the blood clotting cascade have been employed to produce a colored product from a chromogenic substrate as a response. Solid-phase systems have been sped up by the use of ultrasound to enhance the reaction rate of ligand with immobilized binder. Magnetic solid-phase support for antibody has been used to facilitate separation of bound and free fractions in an automated RIA method and in a manual sandwich assay. Bacteriophages have been employed as labels as have chemiluminescent substances (luminol and its derivatives). One method deserving of further comment is the latex agglutination assay, now commonly employed for the rapid diagnosis of infectious agents. Microscopic latex spheres are coated with antibodies (or antigens), and agglutination (visible to the naked eye) is provoked by the presence of the corresponding antigen (or antibody).

Ultrasensitive enzymatic radioimmunoassay (USERIA). There was much excitement in 1979 when Harris et al reported a method of detecting cholera toxin and rotavirus that they claimed was 1000-fold more sensitive than ELISA or RIA. The method combines aspects of radiometric and enzymatic methods (Fig 17–29). A solid-phase antibody is reacted with the analyte/standard, and then a second antibody is added. A third antibody, conjugated to an enzyme (alkaline phosphatase), is then added to the system. Tritiated AMP is then added to the system, and after an incubation period, the tritiated adenosine produced by the enzyme is separated by means of a Sephadex column. The activity of the adenosine is counted and used as response.

Biotin/avidin-enhanced immunoassays. This is another method for which 1000-fold greater sensitivity than RIA has been claimed (Fig 17–30). One of the more straightforward designs requires biotinylation of the binder. This process has a relatively low probability of interfering with binder performance owing to the chemically benign reaction required (formation of amide linkages) and to the low molecular weight (244) of biotin. There may be multiple biotin molecules per binder. The actual label, which may be enzymatic, radioisotopic, fluorometric, metallic, or of another type, is conjugated to avidin. Avidin is a small glycoprotein with an extremely high association constant for reaction with biotin. The label-avidin complex therefore associates with the binder. In another form, this assay employs avidin to bridge biotinylated binder-ligand complexes to a biotinylated label. Large complexes of avidin-biotin-label may also be attached to biotinylated binder. All of these methods are strategies for

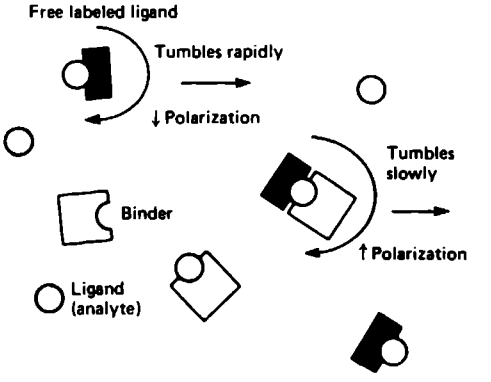


Figure 17–27. FPIA.

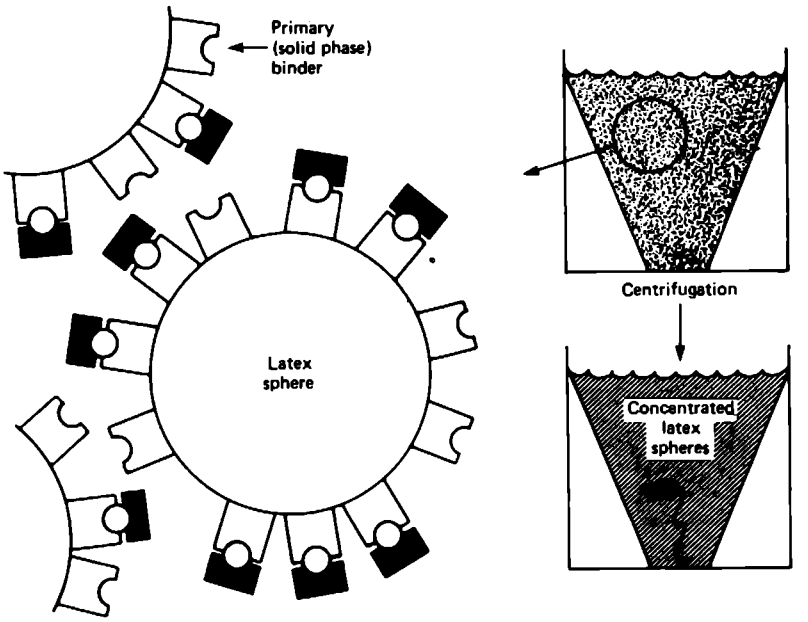


Figure 17–28. PCFIA.

greatly increasing the amount of label per binder molecule.

COMPARING ANALYTIC PERFORMANCE OF DIFFERENT METHODS

The analytic performance of a given method is characterized by that assay's susceptibility to various forms of systematic error, such as the influence of substances that mimic the presence of analyte (**cross-reaction,** which works against assay specificity) or act as another binder or in some other way interfere with truthful measurement (**interference**). It is further characterized by the amount of random error contained in assay results. This is expressed by the confidence limits around an assay determination. Random error determines the **sensitivity** of an assay, by which is meant the lowest amount of analyte that can be statistically distinguished from zero. It is best described in terms of the **lower detection limit** (the analyte concentration for which the confidence interval grazes zero). The precision of the assay, expressed as the width of the confidence interval, can be plotted against concentration to create an **imprecision profile,** which reveals assay error to be nonconstant; it is lowest at some point in mid assay range and increases to either side.

It is difficult to compare the analytic performance of different ligand assay methods, since the workers involved often provide only scanty data, and many new methods are accompanied by exaggerated claims. A rigidly defined standard data reduction method would be of help, so that quantities such as analytic sensitivity and specificity would always be computed in the same manner. An ad hoc international committee convened by the International Atomic Energy Agency has made a start in this direction by describing the basic requirements of a statistically acceptable assay data reduction program. For further information regarding assay terminology, refer to the special glossary of terms provided below.

Although workers tend to compare assay methods according to the labeling method used, Ekins has provided persuasive arguments for classifying methods into 2 basic categories: **limited reagent** (or **competitive) methods** and **excess reagent** (or **noncompetitive) methods.** RIA is an example of the former category; here, labeled ligand and unlabeled ligand compete for a limited amount of binder. The best achievable sensitivity of an RIA is of the order of 10^{-14} mol/L (this represents about $6 \cdot 10^6$ molecules in 1 mL of solution). A sandwich IRMA is an example of the latter category; a massive excess of labeled second antibody is added to the solid-phase binder-ligand complex. Reagent-excess methods are in principle capable of better sensitivity than limited reagent methods, but in practice IRMA has been less sensitive than RIA. ELISA and USERIA have achieved sensitivities one and 2 orders of magnitude smaller than RIA. There are still analytes for which RIA is the only method available, as with vitamin B_{12}, and it seems likely that an array of different methods will remain in use for different applications.

There are alternative methods (such as HPLC) for many analytes. In comparing ligand assay to other measurement methods, an unavoidable limitation of ligand assay must be borne in mind—that it measures

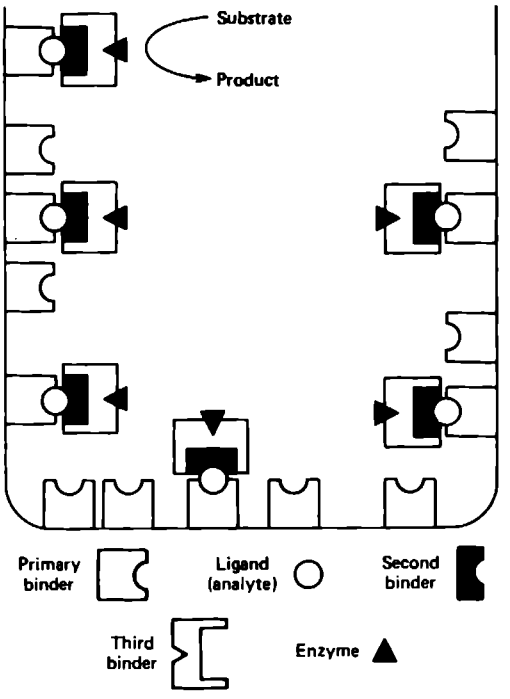


Figure 17–29. USERIA.

chemical (generally immunochemical) reactivity rather than physiologic activity. This has sometimes been turned to advantage, where much new knowledge has been gained about the various forms of drugs and hormones capable of cross-reacting with the intended analyte.

GLOSSARY OF ASSAY TERMINOLOGY

Accuracy: The degree to which an analyte concentration estimate corresponds to the true value, which is rarely knowable absolutely. Inaccuracy is caused by a combination of random and bias errors.

Analyte: The substance to be measured.

Analyte concentration estimate: The concentration of analyte in a test specimen which is estimated by the assay procedure. Also sometimes known as **dose**, a term from the earlier biologic assay field.

Analytic method: The physical and chemical manipulations to which calibrators and test specimens are subjected in order to produce their corresponding assay response values.

Analytic sensitivity: A vague term generally associated with the ability to detect small concentrations but in fact used with multiple meanings, which has provoked immense needless debate. The use of some mathematically defined term such as the **lower detection limit** (see below) is recommended. Analogous to but not to be confused with **diagnostic sensitivity.**

Analytic specificity: A vague term associated with the ability to detect analyte as opposed to nonanalyte. The use of cross-reaction profiles is preferable. Analogous to but not to be confused with **diagnostic specificity.**

Batch: A single processing session in which a set of specimens is subjected to the assay analytic method.

Between-batch random error: The random error that accrues in an assay system, including within-assay random error and the additional sources of random error observed when comparing results obtained from repeated analysis of the same specimen in different batches of the same assay method.

Between-laboratory random error: The random error observed in measurements of the same specimen in different laboratories, including within-assay error, between-batch random error, and the additional sources of random error observed when comparing such results.

Bias: Systematic error. Causes include decay in the performance of the final detection device, specimen misidentification, decay of calibrators or test specimens, nonidentity of the chemical behavior of calibrators and test specimens (as with cross-reaction and interference), and others.

Calibration curve: The plotted or mathematically describable relationship between the response obtained from the analytic procedure and the analyte concentration.

Calibrators: Known concentrations of the analyte, used in creating the calibration curve. Also known as **standards.** There are multiple varieties.

- **Arbitrary calibrator:** Contains an unknown amount of analyte, to which arbitrary units are assigned.
- **Primary calibrator:** Of known composition and high purity.
- **Reference calibrator:** Issued by some central authority to promote standardization of results.
- **Secondary calibrator:** Contains an amount of analyte determined by assay using a primary standard.

Confidence interval: The analyte concentration range contained within a set of confidence limits.

Confidence limits: Statistically defined limits about an analyte concentration estimate, which reflect the random error inherent in the result. Example: 95% limits define a region within which, 95% of the time, the true estimate (in the absence of bias error) would be expected to fall (were the measurement with the accompanying computation of confidence limits to be repeated many times).

Continuous response assay: An assay in which the response varies over a continuous range of values, or a discrete range so large as to be well approximated by a continuous range, as in radioimmunoassay.

Cross-reaction: The reaction with the specific assay binding substance by some chemical entity other

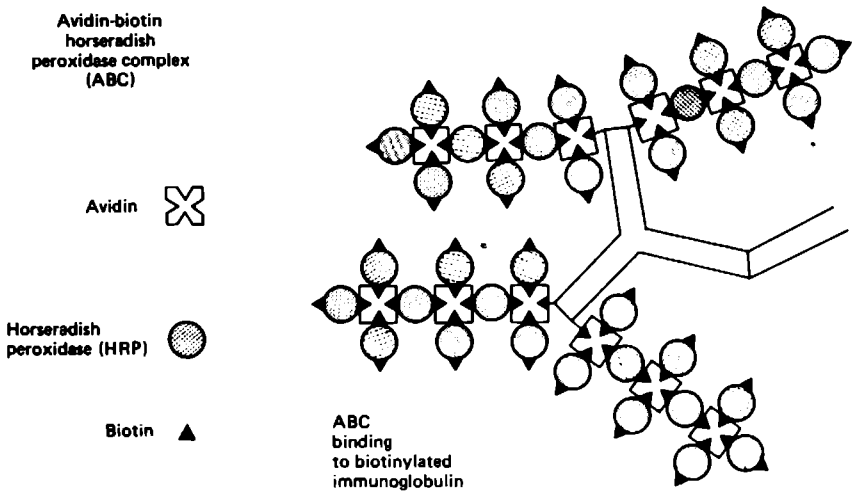


Figure 17–30. Biotin/avidin enhanced assay.

than the analyte, in such a way as to falsify the measured amount of analyte.

Cross-reaction profile: A plot of the bias error in the estimated concentration of analyte as a function of the concentration of a specified cross-reacting species, at a given fixed concentration of analyte.

Cumulative sum chart: A type of quality control chart. The cumulative sum of deviations (from the expected value) of some quality control parameter is plotted. Various statistical tests are available to determine if the plotted points may indicate a quality control problem.

Drift: A temporal shift in bias in assay results. This may sometimes be detected by placing quality control pool specimens at regular intervals in the assay batch.

Imprecision profile: A plot of imprecision (generally expressed as the width of a confidence limit of some specified level of certainty, and a given level of replication of calibrators and unknowns) versus analyte concentration. Also known as a **precision profile.**

Interference: Used in many different ways in clinical chemistry. For example, this term is used to refer to the bias error in a spectrophotometer reading produced by absorption of a given frequency of light by substances other than the one of primary interest. In ligand assay, the term usually refers to the presence of something that can mimic the chemical behavior of the binder, producing bias error. As an example, the presence of thyroid-binding globulin could interfere with a radioimmunoassay of thyroid hormone.

Interpolation: The process of reading the response for an unknown through the calibration curve to obtain an analyte concentration estimate.

Least squares (method of): A procedure for fitting a mathematical function to data in which the sum of the second powers of the vertical distances between the function and the data points is minimized. This method can be broken down into **linear least squares** and **nonlinear least squares,** depending upon whether the equation being fitted is algebraically linear or not. Linear fitting may be done by rather rapid noniterative computational procedures. If the error (in the vertical direction) in the original data is gaussian, estimates for the random error inherent in the parameter estimates can be computed. These will follow gaussian distributions and therefore will be interpretable in probabilistic terms. If nonlinear least squares is employed, an iterative (stepwise) method of solution must be used, and rigorous probabilistic interpretation of the parameter estimates is risky. Appropriate use of the 4-parameter logistic equation requires weighted nonlinear least squares.

Lower detection limit: The (statistically defined) lowest concentration distinguishable from the lowest calibrator concentration (the latter is generally zero). Should be reported along with its associated level of statistical confidence. Also referred to as the **minimum detection limit, minimum detectable dose,** or **lower detection limit.**

Outlier: Defined in 2 senses. First, a member of a replicate set that lies far from the other members of the set, presumably owing to some extraordinary error. Second, the mean of a replicate set for a calibrator that lies far from the calibration curve location suggested by the other calibrators, again presumably owing to some extraordinary error.

Parallelism testing: The process of determining whether the curve that can be drawn from the responses obtained from multiple dilutions of a test specimen can be superimposed upon the calibration curve by a simple multiplicative rescaling of the x axis of the test curve. Also referred to as **similarity testing,** which is perhaps more appropriate, since "parallelism testing" derives from the early days of

biologic assay, when straight-line calibration relationships were employed.

Pooled response-error relationship: A response-error relationship obtained by pooling information from a number of consecutive assay batches (generally 10–30) so as to achieve better estimates of the expected error than would be possible with the limited information available in a single batch.

Power function: Plot of the probability of rejection of an assay result as a function of the amount of error present (either bias or random) for a specified quality control test.

Precision: A qualitative term concerned with the reproducibility of a result and hence with its random error. Avoidance of this term is recommended, preferring the use of some precisely defined mathematic expression of random error, such as a set of confidence limits with its associated level of statistical significance.

Quality control chart: A plot of some assay performance parameter, most often the measurement obtained for a quality control pool, plotted chronologically on a flow chart. Statistical tests may be performed to ascertain if deviations from the average result would be so rare (if attributed to the usual amount of random error of the assay) as to represent a possible quality control problem. Also known as **Shewhart chart** and **Levey-Jennings chart.**

Quality control pool: A large collection of material containing analyte stored so as to preserve it over a long period of time. Aliquots are taken and analyzed (with each assay batch in earlier assays, with decreasing frequency in some newer commercial assays) to assist in detecting exceptional error (particularly useful for bias).

Quantal response assay: An assay in which the response is some value which varies over a discrete range of values (example: 1, 2, 3, ...n) rather than over a continuous range. An example is the type of biologic assay in which a small number of animals is employed and the response is the proportion of animals killed by a given dose of analyte. Requires statistical methods not discussed here.

Random error: Error arising from chance occurrences rather than systematic causes. Important sources in practice include pipetting, timing, and counting and detection errors. See also precision, imprecision, analytic sensitivity, lower detection limit, confidence limits.

Recovery: The amount of analyte detected when a known amount of calibrator is added to a previously assayed test preparation. Significant deviation from 100% suggests the presence of bias error.

Replicate: A repeated analysis of a calibrator, quality control specimen, or unknown, as when a specimen is said to be run in duplicate, triplicate, ...n-tuplicate. The only source of information about random errors.

Residual: The vertical distance between a data point (in assay, this is often the mean of replicates obtained for a given calibrator concentration) and the mathematic equation fitted to it. If the residual is properly weighted for the amount of data it represents and for its variance, it is referred to as a **studentized residual.**

Response: Some mathematic function of the final measurement taken from the assay analytic method.

Response-error relationship: A plot of the error in the selected response versus the response.

Run: Assay jargon for the process of analyzing a single assay batch.

Standard deviation: A measure of the random error inherent in a set of measurements of the same quantity (equal to the square root of the variance). Although the standard deviation may be computed for any given set of numbers, it is interpretable in strict probabilistic terms only if the data are drawn randomly from a gaussian population.

Test specimen: A sample, generally a biologic fluid, an aliquot of which is presented to the assay process in order to determine the concentration of analyte present.

Unknown: Test specimen.

Upper detection limit: The (statistically defined) highest concentration distinguishable from the highest calibrator concentration. Should be reported along with its associated level of statistical confidence.

Valid analytic range;: The range between the upper and lower detection limits.

Variance: A measure of the random error inherent in a set of measurements of the same quantity, equal to the second power of the standard deviation.

Weighting: The use of some estimate of random error in the process of regression so as to take into account the relative error of the data being fitted; the final fit will pay more attention to data of higher precision than to data of lower precision. In assay work, the inverse of the variance (estimated from a pooled response-error relationship) should be employed.

Within-assay random error: The random error observed in assay results analyzed in a single batch.

IMMUNOHISTOCHEMICAL TECHNIQUES

IMMUNOFLUORESCENCE

Immunofluorescence is essentially a histochemical or cytochemical technique for detection and localization of antigens. Specific antibody is conjugated with fluorescent compounds, resulting in a sensitive tracer with unaltered immunologic reactivity. The conjugated antiserum is added to cells or tissues and becomes fixed to antigens, thereby forming a stable immune complex. Nonantibody proteins are removed by

washing, and the resultant preparation is observed in a fluorescence microscope. This adaptation of a regular microscope contains a high-intensity light source, excitation filters to produce a wavelength capable of causing fluorescence activation, and barrier filters to remove interfering wavelengths of light. When observed in the fluorescence microscope against a dark background, antigens bound specifically to fluorescent antibody can be detected by virtue of the bright color of the latter.

The technique of immunofluorescence was introduced in 1941 by Coons, who employed β-anthracene, a blue fluorescing compound, coupled to pneumococcus antiserum to detect bacterial antigens in tissue sections. Shortly thereafter, his group employed fluorescein-conjugated antisera which emitted a green light that could be differentiated from the blue autofluorescence of many tissues.

Fluorescence is the emission of light of one color, ie, wavelength, while a substance is irradiated with light of a different color. The emitted wavelength is necessarily at a lower energy level than the incident or absorbed light (Fig 17–31). Fluorochromes such as rhodamine or fluorescein used in clinical laboratories have characteristic absorption and emission spectra. Fluorescein isothiocyanate (FITC) is a chemical form of fluorescein that readily binds covalently to proteins at alkaline pH primarily through ϵ amino residues of lysine and terminal amino groups. Its absorption maximum is at 490–495 nm, and it emits its characteristic green color at 517 nm. Tetramethylrhodamine isothiocyanate, which emits red, has an absorption maximum at 550 nm and maximal emission at 580 nm (for rhodamine-protein conjugates). Consequently, different excitation and barrier filters must be employed to visualize the characteristic green or red color of these fluorescent dyes. Generally, one wants to achieve an exciting wavelength nearly equal to that of the excitation maximum of the dye. Similarly, the barrier filter should remove all but the emitted wavelength spectrum. In practice, the actual brightness of fluorescence observed by the eye depends on 3 factors: (1) the efficiency with which the dye converts incident light into fluorescent light; (2) the concentration of the dye in the tissue specimen; and (3) the intensity of the exciting (absorbed) radiation.

Microscopes used for visualizing immunofluorescent specimens are simple modifications of standard transmitted light microscopes (Fig 17–32). In 1967, Ploem introduced an epi-illuminated system that employs a vertical illuminator and a dichroic mirror. In this system (Fig 17–33), the excitation beam is focused directly on the tissue specimen through the lens objective. Fluorescent light emitted from the epi-illuminated specimen is then transmitted to the eye through the dichroic mirror. A dichroic mirror allows passage of light of selected wavelengths in one direction through the mirror but not in the opposite direction.

There are several distinct advantages to the Ploem system. Fluorescence may be combined with transmitted light for phase contrast examination of the tissues, thereby allowing better definition of morphology and fluorescence. Also, interchangeable filter systems permit rapid examination of the specimen at different wavelengths for double fluorochrome staining, eg, red and green (rhodamine and fluorescein, respectively). This advantage in technique has resulted in superior sensitivity for examining cell membrane fluorescence in living lymphocytes.

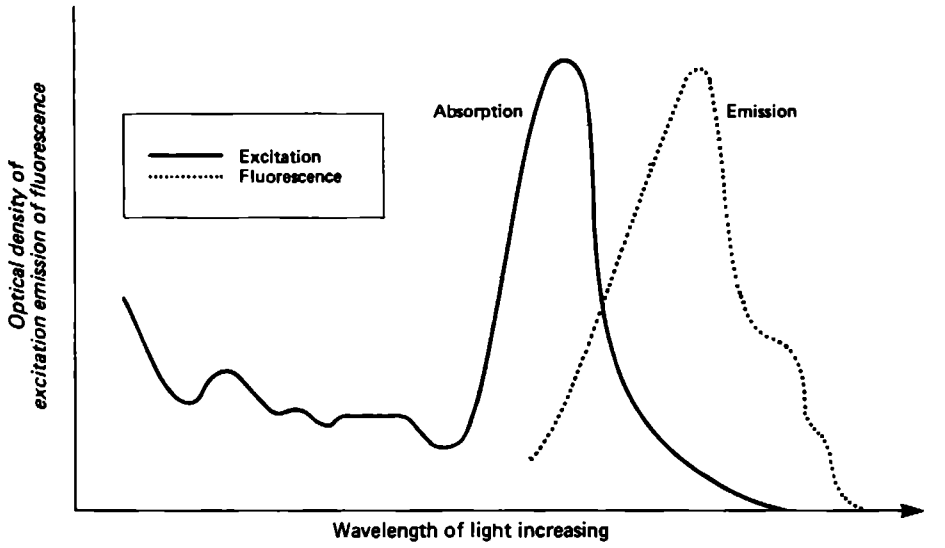


Figure 17–31. Absorption and emission spectra for a fluorescent compound.

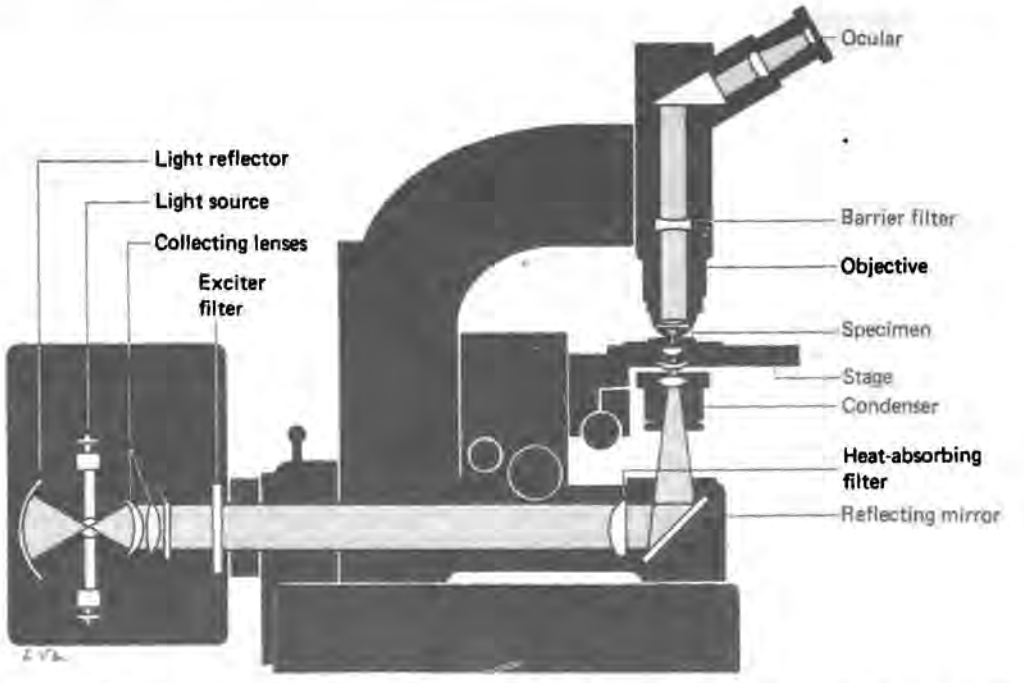


Figure 17–32. Fluorescence microscope with transmitted light. Light beam is generated by a mercury vapor lamp, reflected by a concave mirror, and projected through collecting lenses to the exciter filter, which emits a fluorescent light beam. A reflecting mirror directs the beam from underneath the stage, through the condenser into the specimen. A barrier filter removes wavelengths other than those emitted from the fluorescent compound in the specimen, and the fluorescent pattern is viewed through magnification provided by the objective and ocular lenses.

Methodology & Interpretation

Virtually any antigen can be detected in fixed tissue sections or in live cell suspensions by immunofluorescence. It is the combination of great sensitivity and specificity together with the use of histologic techniques that make immunofluorescence so useful. The steps involved in immunofluorescence include preparation of immune antiserum or purified γ-globulin, conjugation with fluorescent dye, and finally the staining procedure.

For immunofluorescence, an antiserum to the antigen one wishes to detect is raised in heterologous species, eg, goat or rabbit. Potent antisera are needed that should be prepared to contain milligram amounts of antibody per milliliter of antiserum. The potency of antisera is usually assessed by quantitative precipitation or passive hemagglutination. Specificity must be ensured at a level which exceeds that detectable in ordinary double diffusion or immunoelectrophoretic techniques. Several more sensitive methods are available, including hemagglutination inhibition and radioimmunoassay. Unwanted antibodies present in either conjugates or antiglobulin reagents for the test can be removed with insoluble immunoabsorbents.

After one is assured of a direct antiserum of high potency and appropriate specificity, the γ-globulin fraction can be prepared by ammonium sulfate precipitation or a combination of salt precipitation and DEAE-cellulose ion exchange chromatography. It is necessary to partially purify serum immunoglobulin, since subsequent conjugation should be limited to antibody as much as possible. This will increase the efficiency of staining and avoid unwanted nonspecific staining by fluorochrome-conjugated nonantibody serum proteins that can adhere to tissue components.

Conjugation of γ-globulin depends largely on the particular dye one wishes to combine with the antibody molecule. From a clinical standpoint, only fluorescein and rhodamine have been used widely. Fluorescein in the form of FITC, or rhodamine as tetramethylrhodamine isothiocyanate, is either reacted directly with γ-globulin in alkaline solution overnight at 4 °C or dialyzed against γ-globulin. Unreacted dye is then removed from the protein-fluorochrome conjugate by gel filtration or exhaustive dialysis. If necessary, the resultant conjugate can be concentrated by lyophilization, pressure dialysis, or solvent extraction with water-soluble polymers. Thereafter, one must determine both the concentration of γ-globulin and the dye/protein or fluorescein/protein ratio of the compound. This is usually done spectrophotometrically with corrections for the alteration in absorbance of γ-

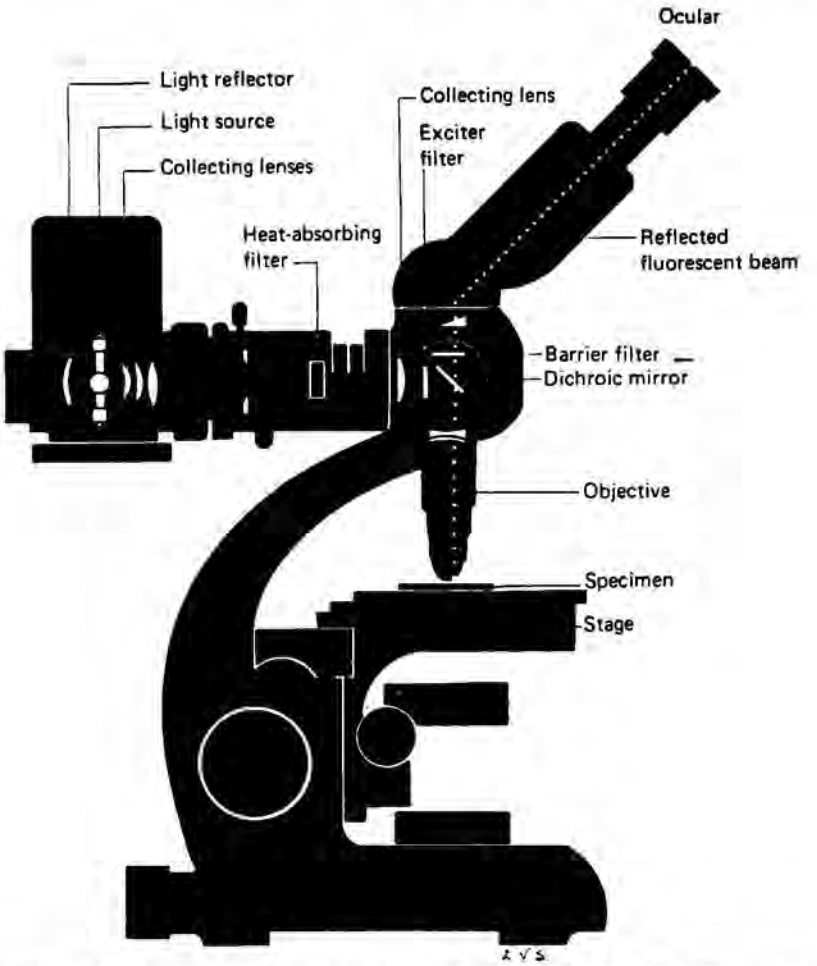


Figure 17–33. Fluorescence microscope with epi-illumination. The light beam is directed through the exciter filter and down onto the specimen. A dichroic mirror allows passage of selected wavelengths in one direction but not another. After reaching the specimen, the light is reflected through the dichroic mirror and emitted fluorescent light is visualized at the ocular.

globulin by the introduced fluorochrome. Fluorochrome-labeled compounds are best stored in the dark, frozen at −20 °C.

Staining Techniques

A. Direct Immunofluorescence: (Figs 17–34 and 17–35.) In this technique, conjugated antiserum is added directly to the tissue section or viable cell suspension.

B. Indirect Immunofluorescence: This technique allows for the detection of antibody in unlabeled sera and is especially useful in the clinical laboratory. It eliminates the need to purify and individually conjugate each serum sample. The method is basically an adaptation of the antiglobulin reaction (Coombs test) or double antibody technique (Figs 17–34 and 17–35). Specificity should be checked as diagrammed and further established by blocking and neutralization methods (Fig 17–36).

Several additional variations in staining techniques have been used. These include a conjugated anticomplement antiserum for the detection of antigen-antibody-complement complexes and double staining with both rhodamine and fluorescein conjugates.

Immunofluorescence employing routine serologic procedures for the detection of antibody in human serum specimens has been widely accepted. Sensitivity levels are generally higher than is the case with complement fixation and lower than with hemagglutination inhibition. Methods for detecting antibody by immunofluorescence include (1) the antiglobulin method, (2) inhibition of labeled antibody-antigen reaction by antibody in test serum, and (3) the anticomplement method.

C. Biotin-Avidin System: Avidin, a basic glycoprotein derived from egg albumin of MW 68,000, has a remarkably high affinity (10^{15} kcal/mol) for the vitamin biotin. Biotin can easily be coupled covalently

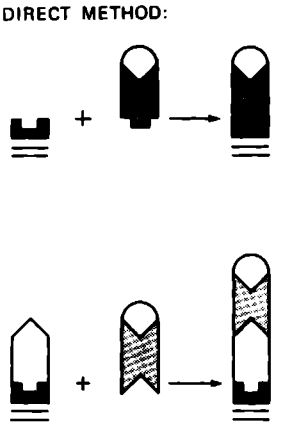


Figure 17–34. Mechanism of immunofluorescence techniques. **Direct Method.** *(Top):* Antigen in substrate detected by direct labeling with fluorescent antibody. *(Bottom):* Antigen-antibody (immune) complex in substrate labeled with fluorescent antiglobulin reagent. (Modified and reproduced, with permission, from Nordic Immunology, Tilburg, The Netherlands.)

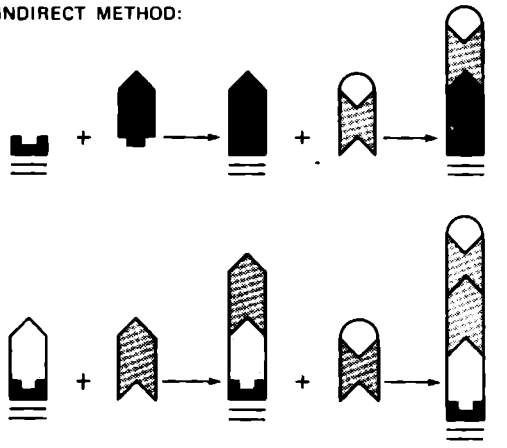
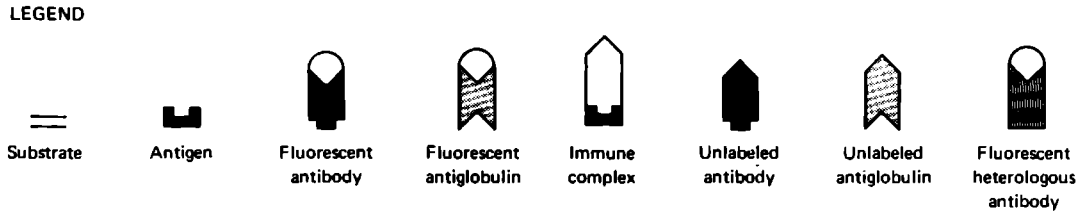


Figure 17–35. Mechanism of immunofluorescence techniques. Indirect Method. *(Top):* Incubation of antigen in substrate with unlabeled antibody forms immune complex. Labeling performed with fluorescent antiglobulin reagent. *(Bottom):* Immune complex in substrate reacted with unlabeled antiglobulin reagent and then stained with fluorescent antiglobulin reagent directed at unlabeled antiglobulin. (Modified and reproduced, with permission, from Nordic Immunology, Tilburg, The Netherlands.)



to a protein (antibody) and then reacted with fluorochrome-coupled avidin. After reaction of antigen with unlabeled antibody, the biotin-labeled second antibody is added. Since many molecules of biotin can be coupled to an antibody, the subsequent addition of fluorochrome-labeled avidin results in a firm bond with exceedingly bright fluorescence. Other advantages are lack of nonspecific binding of fluorochrome-coupled avidin to various substrates and general use of avidin conjugates in binding to biotin-labeled antibodies regardless of their derivation.

Quantitative Immunofluorescence

Quantitative immunoassays using fluorochrome-labeled antigens and antibodies have recently been developed. The amount of light of a given wavelength emitted from a fluorescent specimen can be precisely measured by a microfluorometer. A number of assay methods have been introduced commercially in the field of quantitative immunofluorescence. Fluorescent immunoassay systems can be used to measure IgG, IgA and IgM, C3 and C4, and antinuclear and anti-DNA antibodies. Measurement of immunoglobulins is done by competitive binding of labeled specific antiserum for free and solid phase antigen. The free antigen is present in patients' serum, whereas the bound immunoglobulin is fixed to a polymeric hydrophobic surface. The amount of fluorescent antibody bound to the solid phase antigen is measured in a specially designed microfluorometer and converted to milligrams per deciliter by reference to a standard curve.

Serum antibodies to various cellular antigens such as DNA or nuclei can also be measured by an indirect fluorescence technique. Substrate (eg, DNA) is fixed to the polymer surface in solid phase and incubated with test sera. A second fluorescein anti-immunoglobulin reagent is then bound to the first antigen-antibody complex and the amount of bound fluorescence measured fluorometrically.

This technique has many of the same advantages as enzyme immunoassay, and additional tests are being developed.

Clinical Applications of Immunofluorescence

Direct and indirect immunofluorescence staining techniques have achieved widespread use in many areas of clinical medicine. In general, immunofluores-

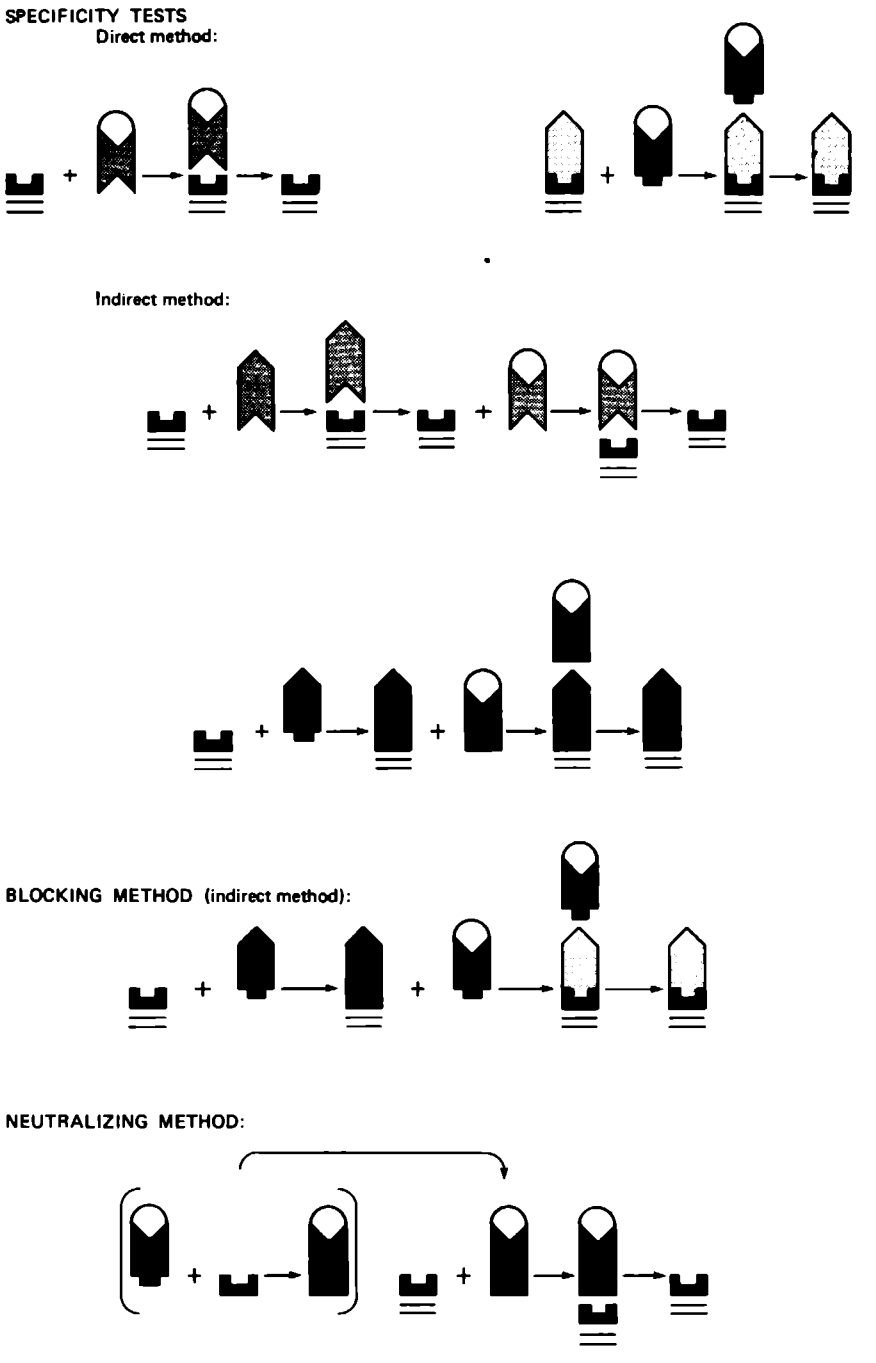


Figure 17–36. Specificity tests. **Direct method.** *(Left):* Substrate antigen fails to react with fluorescent antiglobulin reagent. No fluorescence results. *(Right):* Immune complex-substrate fails to react with fluorescent antibody directed against unrelated antigen. No fluorescence results. **Indirect method.** *(Top):* Unlabeled specific antiglobulin is replaced by unrelated antibody. In second step, fluorescent antiglobulin cannot react directly with antigen in substrate that has not bound specific antiglobulin. No fluorescence results. *(Bottom):* First step performed by reacting specific antibody with substrate antigen. In second stage, the specific conjugate is replaced by unrelated fluorescent heterologous antibody. No fluorescence results. **Blocking method.** Substrate antigen is incubated with unlabeled specific antibody prior to addition of specific fluorescent antibody. Decreased fluorescence results. **Neutralizing method.** Substrate antigen is incubated with specific fluorescent antibody after it is absorbed with specific antigen in substrate. No fluorescence results.

Table 17–8. Clinical applications of immunofluorescence.

Identification of T and B cells in blood
Detection of autoantibodies in serum, eg, ANA
Detection of immunoglobulins in tissues
Detection of complement components in tissues
Detection of specific, tissue-fixed antibody
Rapid identification of microorganisms in tissue or culture
Identification of chromosomes of specific banding patterns
Identification of tumor-specific antigens on neoplastic tissues
Identification of transplantation antigens in various organs
Localization of hormones and enzymes
Quantitation of serum proteins and antibodies

cence has been of greatest usefulness as a sensitive and specific diagnostic tool. A partial list of its applications in clinical immunology is presented in Table 17–8.

OTHER IMMUNOHISTOCHEMICAL TECHNIQUES

Enzyme-Linked Antibody

This method depends on conjugation of an enzyme to antibody that is directed at a cellular or tissue antigen. The resulting conjugate is then both immunologically and enzymatically active. Thereafter, the principles are entirely analogous to those underlying the direct or indirect immunofluorescence techniques described above.

Horseradish peroxidase is usually the enzyme chosen for coupling to antibody. Tissues are first stained directly with antibody-enzyme conjugate or directly with enzyme-linked antiglobulin reagent following incubation with unlabeled immune serum. Thereafter, the tissue is incubated with the substrate for the enzyme. The enzyme in this case is detected visually by formation of a black color after incubation with hydrogen peroxide and diaminobenzidine. One advantage of this method is that ordinary light microscopes may be utilized for analysis of tissue sections. Furthermore, enzyme-coupled antibody can be used for ultrastructural studies in the electron microscope.

A great many additional immunohistochemical techniques have been developed for localization of tissue or cellular antigens. One in particular, the peroxidase-antiperoxidase (PAP) method, has received extensive application in surgical pathology. This is basically a 3-step method. First, fixed slides are stained with rabbit anti-tissue antigen. Next, anti-rabbit immunoglobulin reagent that reacts with the first antibody is applied. Finally, an immune complex consisting of rabbit antiperoxidase-peroxidase attaches to an anti-rabbit antibody bridge. Other techniques being developed include hapten-coupled antibodies, the use of staphylococcal protein A as an intermediate reagent, and systems with more than one immunoenzymatic reagent, ie, double staining. Fixation difficulties and standardization of readily available reagents still limit the wider application of these potentially powerful techniques.

Ferritin-Coupled Antibody

Ferritin, an iron-containing protein, is highly electron-dense. When coupled to antibody, it can be used for either direct or indirect tissue staining. Localization of ferritin-coupled antibody-antigen complexes in fixed tissue can then be achieved with the electron microscope. Other electron-dense particles such as gold or uranium can also be introduced chemically into specific antitissue antibodies. These reagents have also been applied in immunoelectronmicroscopy.

Autoradiography

Radioactive isotopes such as ^{125}I that can be easily introduced into immunoglobulins by chemical means provide highly sensitive probes for localization of tissue antigens. The antigens are detected visually after tissue staining by overlaying or coating slides with photographic emulsion. The appearance of silver grains as black dots has been used for subcellular localization of antigen both at light microscopic and ultrastructural levels. Autoradiography has also been applied to detection of proteins or immunoglobulins synthesized by cells in tissue culture.

Miscellaneous Methods

A variety of other methods have been described for localization of antigens in tissues. Many have not found widespread clinical application. In most cases, these techniques depend on secondary phenomena which occur as a result of the antigen-antibody interaction. These methods include the following:

(1) Complement fixation
(2) Conglutinating complement absorption test
(3) Antiglobulin consumption test
(4) Mixed hemadsorption
(5) Immune adherence
(6) Hemagglutination and coated particle reaction
(7) Immunoprecipitation

AGGLUTINATION

Agglutination and precipitation reactions are the basis of the most commonly used techniques in laboratory immunology. Whereas precipitation reactions are quantifiable and simple to perform, agglutination techniques are only semiquantitative and somewhat more difficult. The agglutination of either insoluble native antigens or antigen-coated particles can be simply assessed visually with or without the aid of a microscope. Important advantages of agglutination reactions are their high degree of sensitivity and the enormous variety of substances detectable through use of antigen- or antibody-coated particles.

According to Coombs, the 3 main requirements in agglutination tests are the availability of a stable cell or particle suspension, the presence of one or more antigens close to the surface, and the knowledge that "incomplete" or nonagglutinating antibodies are not

detectable without modifications, eg, antiglobulin reactions.

Agglutination reactions may be classified as either direct or indirect (passive). In the simple direct technique, a cell or insoluble particulate antigen is agglutinated directly by antibody. An example is the agglutination of group A red blood cells by anti-A sera. Passive agglutination refers to agglutination of antigen-coated cells or inert particles which are passive carriers of otherwise soluble antigens. Examples are latex fixation for detection of rheumatoid factor and agglutination of DNA-coated red cells for detection of anti-DNA antibody. Alternatively, **antigen** can be detected by coating latex particles or red blood cells with purified **antibody** and performing so-called reversed agglutination. Another category of agglutination involves spontaneous agglutination of red blood cells by certain viruses. This viral hemagglutination reaction can be specifically inhibited in the presence of antiviral antibody. Thus, viral hemagglutination can be used either to quantify virus itself or to determine by homologous inhibition the titer of antisera directed against hemagglutinating viruses.

Inhibition of agglutination, if carefully standardized with highly purified antigens, can be used as a sensitive indicator of the amount of antigen in various tissue fluids. Hemagglutination inhibition using passive hemagglutination reactions has recently been semiautomated in microtiter plates and is sensitive for measuring antigens in concentrations of 0.2–9 μg/mL. With appropriate modification, passive hemagglutination with protein-sensitized cells can detect antibody at concentrations as low as 0.03 μg/mL.

AGGLUTINATION TECHNIQUES

Direct Agglutination Test

Red blood cells, bacteria, fungi, and a variety of other microbial species can be directly agglutinated by serum antibody. Tests to detect specific antibody are carried out by serially titrating antisera in 2-fold dilutions in the presence of a constant amount of antigen. Direct agglutination is relatively temperature-independent except for cold-reacting antibody, eg, cold agglutinins. After a few hours of incubation, agglutination is complete and particles are examined either directly or microscopically for evidence of clumping. The results are usually expressed as a titer of antiserum, ie, the highest dilution at which agglutination occurs. Because of intrinsic variability in the test system, a titer usually must differ by at least 2 dilutions ("2 tubes") to be considered significantly different from any given titer. Tests are carried out in small test tubes in volumes of 0.2–0.5 mL or in microtiter plates with smaller amounts of reagents, resulting in greater sensitivity.

Indirect (Passive) Agglutination

The range of soluble antigens that can be passively adsorbed or chemically coupled to red blood cells or other inert particles has dramatically extended the application of agglutination reactions. Many antigens will spontaneously couple with red blood cells and form stable reagents for antibody detection (Table 17–9). When red blood cells are used as the inert particles, serum specimens often must be absorbed with washed, uncoated red blood cells to remove heterophilic antibodies that would otherwise nonspecifically agglutinate the red blood cells. The advantages of using red blood cells for coating are their ready availability, sensitivity as indicators, and storage capabilities. Red blood cells can be treated with formalin, glutaraldehyde, or pyruvic aldehyde and stored for prolonged periods at 4 °C. Although not applicable to all coating antigens, treatment with these preservatives may often be performed either before or after antigen coupling.

Coupling techniques vary greatly in their applicability and success in individual laboratories. A list of general methods available for coating antigens on red blood cells is presented in Table 17–10. Perhaps the most widely used method is the tanned cell technique. Treatment of red blood cells with tannic acid increases the amount of most protein antigens subsequently adsorbed. This higher density of coated antigen greatly increases the sensitivity of the agglutination reaction. Although highly purified antigens are required for immunologic specificity, slightly denatured or aggregated antigens coat tanned red blood cells best.

Agglutination tests may be performed in tubes or microtiter plates. In antisera with very high agglutination titers, a prozone phenomenon may obscure the results. The prozone phenomenon produces falsely negative agglutination reactions at high concentrations of antibody as a result of poor lattice formation and steric hindrance by antibody excess. However, the use of standard serial dilutions eliminates this difficulty. Since IgM antibody is about 750 times as efficient as IgG in agglutination, the presence of high amounts of IgM may markedly influence test results.

HEMAGGLUTINATION INHIBITION

The inhibition of agglutination of antigen-coated red blood cells by homologous antigen is a highly sensitive and specific method for detecting small quantities of soluble antigen in blood or other tissue fluids. The principle of this assay is that antibody preincubated with soluble homologous or cross-reacting antigens will be "inactivated" when incubated with antigen-coated red blood cells. Thus, the test proceeds in 2 stages (Fig 17–37). Antibody in relatively low concentration is incubated with a sample of antigen of unknown quantity. After combination with soluble antigen, antigen-coated cells are added and agglutinated by uncombined or free antibody. (The degree of inhibition of agglutination reflects the amount of antigen present in the original sample.) Controls, including

Table 17–9. Substances that spontaneously adsorb to red blood cells for hemagglutination.

Escherichia coli antigens
Yersinia antigens
Lipopolysaccharide from *Neisseria meningitidis*
Toxoplasma antigens
Purified protein derivative (PPD)
Endotoxin of *Mycoplasma* species
Viruses
Antibiotics, especially penicillin
Ovalbumin
Bovine serum albumin
DNA
Haptens, eg, DNCB

samples of known antigen concentration and uncoated red blood cells, must be employed. This hemagglutination inhibition method has proved very useful in the detection of HBsAg in hepatitis and in the detection of factor VIII antigen in hemophilia and related clotting disorders.

CLINICALLY APPLICABLE TESTS THAT EMPLOY AGGLUTINATION REACTIONS

Antiglobulin Test (Coombs Test)

The development of this simple and ingenious technique virtually revolutionized the field of immunohematology, and in various forms it has found widespread application in all fields of immunology. Antibodies frequently coat red blood cells but fail to form the necessary lattice which results in agglutination. A typical example is antibody directed at the Rh determinants on human red blood cells. However, the addition of an antiglobulin antiserum produced in a heterologous species (eg, rabbit anti-human γ-globulin) produces marked agglutination. Thus, the antiglobulin or Coombs test is used principally to detect subagglutinating or nonagglutinating amounts of anticrythrocyte antibodies of any γ-globulin molecule. However, more specific Coombs reagents directed at specific immunoglobulin classes, eg, anti-IgG, anti-IgA, or anti-L chains, may also be employed to detect cell-bound immunoglobulin. So-called non-gamma Coombs reagents which are directed against various complement components, eg, C3 or C4, may also produce red cell agglutination in the case of autoimmune hemolytic anemia. In some instances of this disorder, only complement components are bound to the red cell and the classic antiglobulin reaction is negative. The **direct Coombs test** detects γ-globulin or other serum proteins that are adherent to red blood cells taken directly from a sensitized individual. The **indirect Coombs test** is a 2-stage reaction for detection of incomplete antibodies in a patient's serum. The serum in question is first incubated with test red blood cells, and the putative antibody-coated cells are then agglutinated by a Coombs antiglobulin serum. The major applications of Coombs tests include red cell typing in blood banks, the evaluation of hemolytic disease of the newborn, and the diagnosis of autoimmune hemolytic anemia.

Table 17–10. Methods used to coat fresh and aldehyde-treated red blood cells with various antigens and antibodies for passive hemagglutination assay.*

Coupling Agent	Comments on Coupling	Antigens Commonly Coated
None	Simple adsorption	Penicillin, bacterial antigens including endo- and exotoxins, viruses, and ovalbumin.
Tannic acid	Adsorption possibly caused by changes analogous to enzymes. Most popular; usually satisfactory, but often difficult and unreliable.	A wide spectrum of antigens: serum proteins, microbial and tissue extracts, homogenates, thyroglobulin, and tuberculin proteins.
Bisdiazotized benzidine (BDB)	Chemically stable covalent azo bonds.	Proteins and pollen antigens.
1,3-Difluoro-4, 6-dinitrobenzene (DFDNB)	Adsorption after modification of cell membrane.	Purified proteins and chorionic gonadotropin.
Chromic chloride ($CrCl_3$)	Proteins bound to red cells by the charge effect of trivalent cations.	Proteins.
Glutaraldehyde, cyanuric chloride, tetrazotized O-dianisidine	Cross-linking and covalent coupling.	Various proteins and certain enzymes.
Tolylene-2,4-diisocyanate	Covalently bound.	Proteins.
Water-soluble carbodiimide	Covalently bound.	Proteins.

*Modified and reproduced, with permission, from Fudenberg HH: Hemagglutination inhibition: Passive hemagglutination assay for antigen-antibody reactions. In: *A Seminar on Basic Immunology*. American Association of Blood Banks, 1971.

Bentonite Flocculation Test

Passive carriers of antigen other than red blood cells have been widely used in serology for the demonstration of agglutinating antibody. Wyoming bentonite is a form of siliceous earth that can directly adsorb most types of protein, carbohydrate, and nucleic acid. After adsorption, many antigens are stable on bentonite for 3–6 months. Simple flocculation on slides with appropriate positive and negative control sera indicates the presence of serum antibody. Bentonite flocculation has been employed to detect antibodies to *Trichinella*, DNA, and rheumatoid factor.

Latex Fixation Test

Latex particles may also be used as passive carriers for adsorbed soluble protein and polysaccharide antigens. The most widespread application of latex agglu-

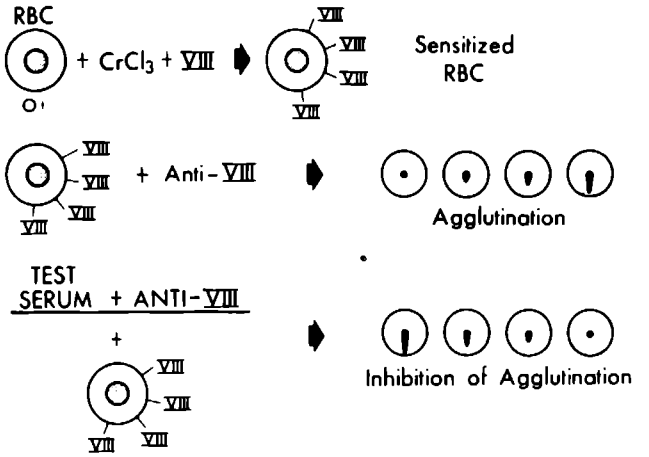


Figure 17–37. Hemagglutination inhibition. Human O + red blood cells (RBC) are conjugated with coagulation factor VIII antigen by chromic chloride. The sensitized red blood cells are reacted with specific antibody to factor VIII and are agglutinated. In the well of a V-shaped microtiter plate, agglutinated red blood cells appear as discrete dots. Nonagglutinated cells form a streak when the plate is incubated at a 45-degree angle. Agglutination of sensitized red blood cells can be inhibited by the presence of homologous factor VIII antigen present in the test serum. With decreasing amounts of serum added to the test, the specific antibody agglutinates sensitized cells and forms a dot in the microtiter well. A semiquantitative estimation of the amount or titer of antigen in a test serum can be made in this way.

tination (fixation) has been in the detection of rheumatoid factor. Rheumatoid factor is a 19S IgM antibody directed against 7S IgG (see Chapter 21). If 7S IgG is passively adsorbed to latex particles, specific determinants on the IgG are revealed which then react with IgM rheumatoid factors. This method is more sensitive but less specific for rheumatoid factor than the Rose-Waaler test (see below). Latex fixation has also been used to detect urine hCG in pregnancy testing.

Rose-Waaler Test

This passive hemagglutination test is also used for the detection of rheumatoid factor. Tanned red blood cells (usually sheep) are coated with subagglutinating amounts of rabbit 7S IgG antibodies specific for sheep red blood cells. Human rheumatoid factor will agglutinate these rabbit immunoglobulin-sensitized sheep red blood cells by virtue of a cross-reaction between rabbit IgG and human 7S IgG. The use of this test and latex fixation in the diagnosis of rheumatoid diseases (especially rheumatoid arthritis) is discussed in Chapter 21.

COMPLEMENT FUNCTION

Complement is one of the main humoral effector mechanisms of immune complex-induced tissue damage (see Chapters 10 and 12). Clinical disorders of complement function have been recognized for many decades, but their mechanism and eventual treatment have awaited elucidation of the complement sequence itself. The 9 major complement components (C1–C9) and various inhibitors can now be measured in human serum. Clinically useful assays of complement consist primarily of CH_{50} or total hemolytic assay and specific functional or immunochemical assays for various components. Immunochemical means are available for determining serum concentrations of selected components, but these assays do not provide data regarding the functional integrity of the various molecules.

It is worth emphasizing that the collection and storage of serum samples for functional or immunochemical complement assays present special problems as a result of the remarkable lability of some of the complement components. Rapid removal of serum from clotted specimens and storage at temperatures of −70 °C or lower are required for preservation of maximal activity.

Finally, complement fixation or utilization, which occurs as a consequence of the antigen-antibody reaction, provides a sensitive and highly useful means of detecting antigens or antibodies and has been of particular use in serology.

HEMOLYTIC ASSAY

Specific antibody-mediated hemolysis of erythrocytes in the presence of the intact complement sequence can be used as a crude screening test for complement activity in human serum. However, it has limited usefulness, since a drastic reduction in components is necessary to produce a reduction in the hemolytic assay. Classically, the hemolytic assay employs sheep erythrocytes (E), rabbit antibody (A) to sheep red blood cells, and fresh guinea pig serum as a

source of complement (C). Hemolysis is measured spectrophotometrically as the absorbance of released hemoglobin and can be directly related to the number of red blood cells lysed. The amount of lysis in a standardized system employing E, A, and C describes an S-shaped curve when plotted against increasing amounts of added complement (Fig 17–38).

The curve is S-shaped, but in the mid region, near 50% hemolysis, a nearly linear relationship exists between the degree of hemolysis and the amount of complement present. In this range, the degree of red blood cell lysis is very sensitive to any alteration in complement concentration. For clinical purposes, measurement of total hemolytic activity of serum is taken at 50% hemolysis level. The CH_{50} is an arbitrary unit defined as the quantity of complement necessary for 50% lysis of red cells under rigidly standardized conditions of red blood cell sensitization with antibody (EA). These results are expressed as the reciprocal of the serum dilution giving 50% hemolysis. Many test variables can influence the degree of hemolysis. These include red cell concentration, fragility (age) of red blood cells, amount of antibody used for sensitization, nature of antibody (eg, IgG or IgM), ionic strength of the buffer system, pH, reaction time, temperature, and divalent cation (Ca^{2+} or Mg^{2+}) concentrations.

The value for CH_{50} units in human serum may be determined in several ways. Usually, one employs the von Krogh equation, which converts the S-shaped complement titration curve into a nearly straight line.

The S-shaped curve in Fig 17–38 is described by the von Krogh equation:

$$X = K\left(\frac{Y}{1-Y}\right)^{1/n}$$

where X = mL of diluted complement added,
Y = degree of percentage lysis,
K = constant,
n = 0.2 ± 10% under standard E and A conditions.

It is convenient to convert the von Krogh equation to a log form that renders the curve linear for plotting of clinical results (Fig 17–39):

$$\log X = \log K + \frac{1}{n}\log\frac{Y}{1-Y}$$

The values of $Y/1-Y$ are plotted on a log-log scale against serum dilutions. The reciprocal of the dilution of serum that intersects the curve at the value $Y/1-Y = 1$ is the CH_{50} unit. Values for normal CH_{50} units vary greatly depending on particular conditions of the test employed. It should again be emphasized that the CH_{50} (hemolytic) assay is relatively insensitive to reduction in specific complement components and may in fact be normal or only slightly depressed in the face of significant reductions in individual components.

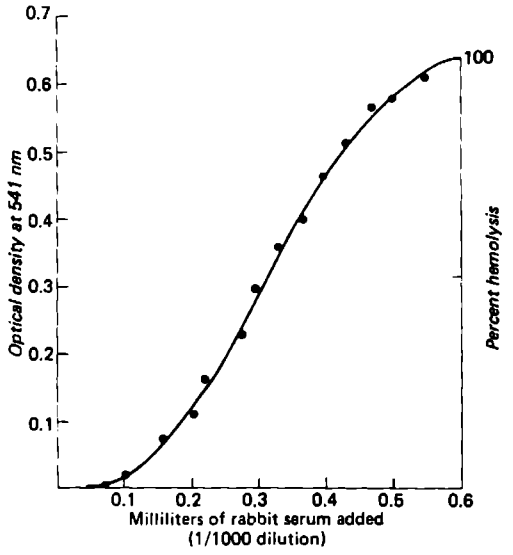


Figure 17–38. Relationship of complement concentration and red blood cells lysed. Curve relating the percentage of hemolysis that results from increasing amounts of fresh rabbit serum (diluted 1:1000) as complement source is added to sensitized sheep red blood cells (erythrocyte amboceptor [EA]). Hemolysis can be precisely determined by measuring the optical density of hemolysis supernates at 541 nm, the wavelength for maximal absorbance by hemoglobin.

MEASUREMENT OF INDIVIDUAL COMPLEMENT COMPONENTS

Functional Assays

Activation of the entire complement sequence of C1–C9 must occur to produce lysis of antibody-coated erythrocytes (EA). Thus, a general scheme can be proposed to determine the level of activity of individual complement components. Initially, one must obtain pure preparations of each of the individual components. These pure components are then added sequentially to EA until the step is reached just prior to the component to be measured. The test sample is added and the degree of subsequent erythrocyte lysis is then related to the presence of the later-acting components. Of course, all proximal components must be supplied in excess in order to measure more distally acting intermediates. Alternatively, the presence of genetically defined complement deficiencies has made available to the laboratory a further source of specifically deficient reagents for estimating individual component activity. A description of the technique of functional assays for complement components is found in the monograph of Rapp and Borsos.

Immunoassays for Complement Components

Antibodies can be prepared against most of the major complement components, thereby allowing for

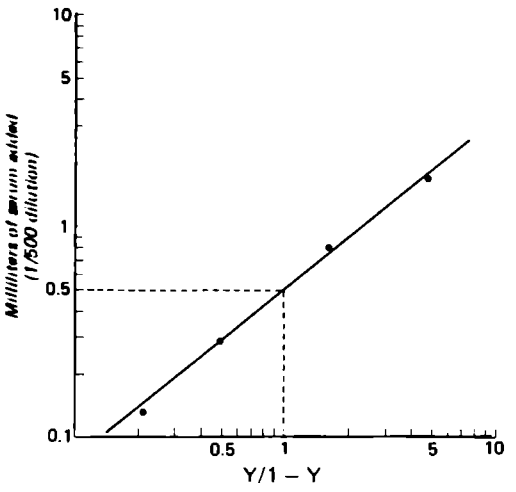


Figure 17–39. Determination of CH_{50} units from serum. Standard curve relating milliliters of serum 1:500 dilution to Y 1 –Y from von Krogh equation. When Y/1 –Y = 1.0, the percentage of lysis equals 50%. In the example shown, 0.5 mL of 1:500 serum dilution has produced Y/1 –Y = 1.0, or 50% lysis. The CH_{50} value for this serum equals 1000, since 1 mL of serum would have 1000 lytic units.

simple and precise immunochemical determinations of complement components. Techniques that have been utilized for this purpose include electroimmunodiffusion (Laurell rocket electrophoresis), single radial diffusion, and quantitative immunofluorescence. Although immunologic assay of complement components is independent of their biologic function, alterations in chemical composition of complement components during storage may alter their behavior in these immunoassays. For example, in storage, C3 spontaneously converts to C3c, which has a smaller molecular size than native C3. Thus, when single radial diffusion of timed interval variety is used, stored serum will give falsely high estimates because more rapid diffusion produces a larger ring diameter. Crucial to accuracy in clinical laboratory tests for complement is reliability of standards. In general, commercial sera prepared from large normal donor pools are adequate. However, since complement components are thermolabile when stored above −70 °C, great care must be taken to ensure adequate refrigerated storage. In fact, the major source of error in complement determination is poor sample handling.

Significance of CH_{50} Units

A. Reduced Serum Complement Activity: Reduced amounts of serum complement activity have been reported in a variety of disease states (Table 17–11). The reduction in serum complement activity could be due to any one or a combination of (1) complement consumption by in vivo formation of antigen-antibody complexes, (2) decreased synthesis of complement, (3) increased catabolism of complement, or (4) formation of an inhibitor. Although complement has been demonstrated fixed to various tissues, eg, glomerular basement membrane, in association with antibody, tissue fixation of complement is apparently not an important mechanism in lowering serum complement activity. In addition, hemolytic activity (CH_{50}) may be relatively unaffected by major changes in concentration of individual complement components. Isolated reduction in human serum levels of C1, C2, C3, C6, or C7 to 50% of normal only slightly reduces hemolytic activity. For this reason, many laboratories have switched from classic hemolytic assay to a more simple immunochemical determination of C3. In general, the reduction of C3 correlates positively with CH_{50} activity reduction.

Table 17–11. Diseases associated with reduced hemolytic complement activity.

Systemic lupus erythematosus with glomerulonephritis
Acute glomerulonephritis
Membranoproliferative glomerulonephritis
Acute serum sickness
Immune complex diseases
Advanced cirrhosis of the liver
Disseminated intravascular coagulation
Severe combined immunodeficiency
Infective endocarditis with glomerulonephritis
Infected ventriculoarterial shunts
Hereditary angioneurotic edema
Hereditary C2 deficiency
Paroxysmal cold hemoglobinuria
Myasthenia gravis
Infective hepatitis with arthritis
Allograft rejection
Mixed cryoglobulinemia (IgM-IgG)
Lymphoma

B. Elevated Complement Levels: Although complement levels are elevated in a variety of diseases (Table 17–12), the significance of this observation is not clear. The most likely mechanism is overproduction.

The development of specific functional and immunologic methods for detecting complement components has led to the discovery of a variety of genetically determined disorders of the complement system. A discussion of the specific disease states which result from selective deficiency of the various complement components is found in Chapter 20.

Table 17–12. Diseases associated with elevated serum complement concentrations.

Obstructive jaundice
Thyroiditis
Acute rheumatic fever
Rheumatoid arthritis
Polyarteritis nodosa
Dermatomyositis
Acute myocardial infarction
Ulcerative colitis
Typhoid fever
Diabetes
Gout
Reiter's syndrome

C. Abnormalities of the Alternative Pathway of Complement: Conceivably, either deficiencies or excesses in amount or function of the 6 plasma proteins of the alternative pathway could lead to disease. Clinically relevant abnormalities in this system are so rare that testing for their components is usually of little value. A few patients with increased bacterial infection and inherited deficiencies of C3b or factor B have been described. Possible abnormalities in paroxysmal nocturnal hemoglobinuria, membranoproliferative glomerulonephritis, and some immune complex diseases are currently being investigated.

COMPLEMENT FIXATION

The fixation of complement occurs during the interaction of antigen and antibodies. Thus, the consumption of complement in vitro can be used as a test to detect and measure antibodies, antigens, or both. The test depends on a 2-stage reaction system. In the initial stage, antigen and antibody react in the presence of a known amount of complement and complement is consumed (fixed). In the second stage, hemolytic complement activity is measured to determine the amount of complement fixed and thus the amount of antigen or antibody present in the initial mixture (Fig 17–40). The amount of activity remaining after the initial antigen-antibody reaction is back-titrated in the hemolytic assay (see above). Results are expressed as either the highest serum dilution showing fixation for antibody estimation or the concentration of antigen that is limiting for antigen determinations.

Extremely sensitive assays for antigen or antibody concentrations have been developed using microcomplement fixation. However, these assays are too cumbersome and complex for routine clinical laboratory use.

Complement Fixation Tests

Complement fixation tests (Fig 17–40) have received widespread application in both research and clinical laboratory practice. Table 17–13 lists some of the applications of complement fixation for either antigen or antibody determination. It should be recalled that all complement assay systems involving functional tests can be inhibited by anticomplementary action of serum. This may result from antigen-antibody complexes, heparin, chelating agents, and aggregated immunoglobulins, eg, as in multiple myeloma.

MONOCLONAL ANTIBODIES

The production of monoclonal antibodies by somatic cell hybridization of antibody-forming cells and continuously replicating cell lines has created a revolution in immunology. The technique of hybridoma formation described by Köhler and Milstein in 1975 has allowed immunologists to prepare virtually unlimited quantities of antibodies that are chemically, physically, and immunologically completely homogeneous. These molecules are then generally unencumbered by nonspecificity and cross-reactivity. Many examples of their potential and current uses in immunology have been discussed (see Chapter 11). In

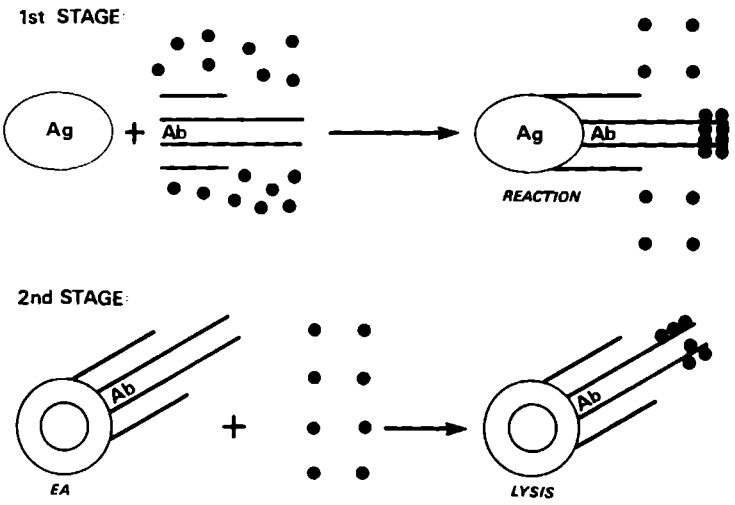


Figure 17–40. Principles of complement fixation. In the first stage, antigen (Ag) and antibody (Ab) are reacted in the presence of complement (●). The interaction of Ag and antibody fixes some but not all of the complement available. In the second stage, the residual or unfixed complement is measured by adding EA (erythrocyte amboceptor), which is lysed by residual complement. Thus, a reciprocal relationship exists between amounts of lysis in second stage and antigen present in the first stage.

Table 17–13. Applications of complement fixation tests.

Hepatitis-associated antigen (HBsAg)
Antiplatelet antibodies
Anti-DNA
Immunoglobulins
L chains
Wassermann test for syphilis
Coccidioides immitis antigen

laboratory immunology, monoclonal antibodies are already being made to detect cellular and soluble antigens with RIA, ELISA, and IFA. Some well-established immunochemical methods such as immunodiffusion and immunoelectrophoresis probably do not require the degree of specificity afforded by monoclonal antibodies.

Technique of Monoclonal Antibody Production (Fig 17–41)

Hybridomas or somatic cell hybrids can readily be formed by fusing a single cell suspension of splenocytes or lymphocytes from immunized mice or rats to cells of continuously replicating tumor cells, eg, myelomas or lymphomas. The replicating cell line is selected for 2 distinct properties: (1) lack of immunoglobulin production or secretion, and (2) lack of hypoxanthine phosphoribosyl transferase (HPRT) activity. The cells are fused by rapid exposure to polyethylene glycol. Thereafter, 3 cell populations remain in culture: splenocytes, myeloma cells, and hybrids. The hybrids have the combined genome of the parent lines and eventually extrude chromosomes and acquire a diploid state. Selection for the hybrids is accomplished by awaiting natural death of the splenocytes. The myeloma cell line is killed, because in HAT medium, which contains hypoxanthine, aminopterin, and thymidine, HPRT cells cannot use exogenous hypoxanthine to produce purines. Aminopterin blocks endogenous synthesis of purines and pyrimidines, and the cells die. Hybrids begin to double every 24–48 hours, and colonies rapidly form.

The hybridoma cells are then cloned by limiting dilution methods and supernates assayed for antibody production, usually by ELISA or RIA. Recloning is performed to ensure monoclonality, and large numbers of cells are grown for antibody production. Extensive immunochemical and serologic studies are performed to ensure antibody specificity. Production of large quantities of antibody can be done in tissue culture or by ascites formation in syngeneic mice. Storage of cells in liquid nitrogen for further use is easily accomplished.

Inter- as well as intraspecies heterokaryons can be produced and propagated in long-term culture. In human therapeutic research, mouse hybridomas have usually been employed, but these regularly produce anti-murine immunoglobulin responses after infusions in humans. For this reason and for maximum specificity, human-to-human hybridomas have also been developed. Limitations in range of antibody

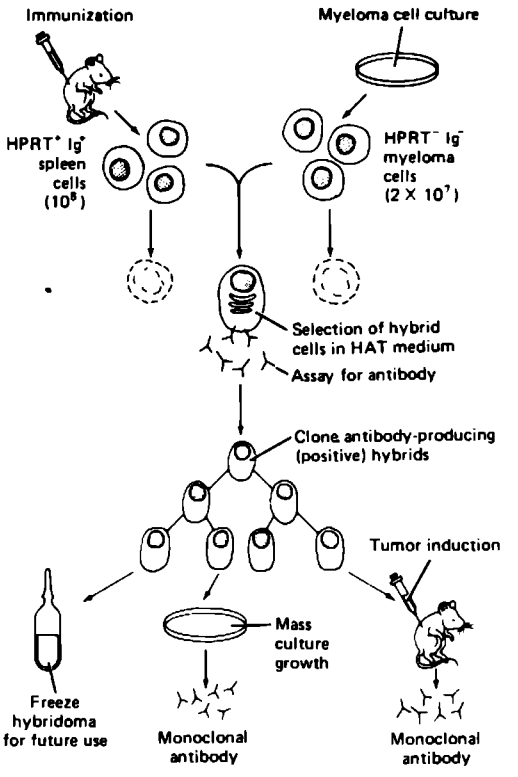


Figure 17–41. Formation of hybridomas between mouse cells and myeloma cells. Mouse myeloma cells that do not produce their own immunoglobulins and lack hypoxanthine phosphoribosyl transferase (HPRT) are fused to splenocytes from an immunized mouse with polyethylene glycol. The hybrid cells are selected in hypoxanthine-aminopterin-thymidine (HAT) medium. Unfused myeloma cells are killed by HAT and unfused splenocytes die out. The hybridomas are cloned, and antibody is produced in tissue culture or by ascites formation. (Reproduced, with permission, from Diamond BA, Yelton DE, Scharff MD: Monoclonal antibodies: A new technique for producing serologic reagents. *N Engl J Med* 1981;304:1344.)

specificities and technical difficulties in maintaining these hybridomas in culture need to be overcome. Since many if not all human B cell lines contain Epstein-Barr virus, this agent probably needs to be eliminated or inactivated prior to potential therapeutic use of these cultures or their products.

Some examples of application of monoclonal antibodies in immunology are listed in Table 17–14.

COMPARATIVE SENSITIVITY OF QUANTITATIVE IMMUNOASSAYS

A major limitation of all quantitative immunoassays is their sensitivity. Exact lower limits of sensitivity vary with avidity, concentration, lots of an-

Table 17–14. Applications of monoclonal antibodies.

Diagnostic (Many achieved; some experimental.)
Leukocyte identification
Lymphocyte subset determination
HLA antigen detection
Individual specificities of A, B, C, DR loci
Framework specificities
Viral detection and subtyping, eg, influenza variants
Parasite identification
Other microbe detection
Polypeptide hormone detection
Relatedness of hormones, eg, hGH, hCS, hPRL
Detecting carcinoembryonic protein, eg, CEA, AFP
Detecting cardiac myosin for myocardial injury
Typing leukemias and lymphomas
Detecting tumor-related antigens
Monitoring immunotherapy in many diseases
Immunohistochemical application in tissue sections
Therapeutic (Experimental; can be coupled to a toxin or radioisotope to enhance in vivo effects.)
Antitumor therapy
Individual tumor antigen-specific
Anti-idiotype to surface immunoglobulin B cell lymphomas
Immunosuppression
Organ transplantation
Autoimmune and hypersensitivity diseases
Treatment of GVH disease
Fertility control
Anti-hCG or antitrophoblast antibodies
Drug toxicity reversal, eg, digitalis intoxication
(All carry possible risk of transmitting microbial diseases from contaminants.)

Table 17–15. Relative sensitivity of assays for antigens and antibodies.*

Technique	Approximate Sensitivity (per dL)
Total serum proteins (by biuret or refractometry)	100 mg
Serum protein electrophoresis (zone electrophoresis)	100 mg
Analytic ultracentrifugation	100 mg
Immunoelectrophoresis	5–10 mg
Immunofixation	5–10 mg
Single radial diffusion	<1–2 mg
Double diffusion in agar (Ouchterlony)	<1 mg
Electroimmunodiffusion (rocket electrophoresis)	<0.5 mg
One-dimensional double electroimmunodiffusion (counterimmunoelectrophoresis)	<0.1 mg
Nephelometry	0.1 mg
Complement fixation	1 μg
Agglutination	1 μg
Enzyme immunoassay	<1 μg
Quantitative immunofluorescence	<1 pg
Radioimmunoassay	<1 pg

*Modified and reproduced, with permission, from Ritzmann SE: *Behring Diagnostics Manual on Proteinology and Immunoassays*, 2nd ed. Behring Diagnostics (New Jersey), 1977.

tisera, temperature, length of reaction, and other individual laboratory practices. Nevertheless, it is useful to consider the approximate limits of sensitivity of various methods available in the clinical immunology laboratory. The most commonly employed techniques are listed in Table 17–15 in order of increasing sensitivity.

PREDICTIVE VALUE THEORY & IMMUNOLOGIC TESTING

When *any* test is used to make a decision, there is some probability of drawing an erroneous conclusion. Increased awareness of this problem has led to the growth in recent years of a discipline known as predictive value theory, which is an early and relatively simple component of a rapidly developing discipline known as **decision theory.** An example of its application in the context of the medical diagnosis of multiple sclerosis follows. Although this diagnosis is still made by using the patient's history and physical findings, several laboratory tests have been put forward as decision aids. One study has presented figures for several such tests, including the CSF IgG index, which is a ratio of ratios, [CSF IgG:albumin]:[serum IgG:albumin]. To simplify discussion, we have consolidated the patients in this study into 2 groups (those with definite or probable multiple sclerosis, and all others).

For every individual in this study, there are 2 items of information: the diagnostic category (disease or no disease) and the result of the laboratory test (normal or abnormal). This divides the results into 4 categories: **true positives** and **true negatives,** and **false positives** and **false negatives.** It is the presence of false-positive and false-negative results that leads to erroneous conclusions. The results can be presented as a two-by-two contingency table as in Table 17–16.

We can now define the basic quantities used by predictive value theory. **Diagnostic sensitivity** (not to be confused with analytic sensitivity, discussed in the context of ligand assay) is the fraction of diseased subjects with abnormal test results. **Diagnostic specificity** is the fraction of nondiseased subjects who have a normal laboratory test. The **positive predictive value** is the fraction of abnormal tests that represent disease, and **negative predictive value** is the fraction of normal tests that represent the absence of disease. Computing these values for the data in Table 17–16, we find

$$\text{Sensitivity} = \frac{54}{64} = 0.84$$

$$\text{Specificity} = \frac{110}{129} = 0.85$$

$$\text{Positive predictive value} = \frac{54}{73} = 0.74$$

$$\text{Negative predictive value} = \frac{110}{120} = 0.92$$

Table 17–16. A two-by-two contingency table.

Test Status	Disease Status		Totals
	Present	Absent	
Positive	54 (True positives)	19 (False positives)	73
Negative	10 (False negatives)	110 (True negatives)	120
Totals	64	129	193

Table 17–17. The effect of prevalence upon predictive value.

Test Status	Disease Status		Totals
	Present	Absent	
Positive	54 (True positives)	280 (False positives)	334
Negative	10 (False negatives)	1586 (True negatives)	1596
Totals	64	1866	1930

Note that diagnostic sensitivity and specificity reveal something about the test *given prior knowledge about the disease status*, while positive and negative predictive values estimate the *likelihood of disease given the test result*. Clearly, it is the latter case which is of interest when trying to make a diagnosis. In this context, it is vital to realize that while diagnostic sensitivity and specificity are qualities of a test, positive and negative predictive values are determined by both test performance and the prevalence of the disease in the patient population under study. Prevalence is defined as the proportion of the population which is afflicted by the disease in question. For the patient population studied in Table 17–16, the prevalence was (64/193 = 0.33), or 33%. Table 17–17 illustrates the effect of prevalence by presenting data for the CSF index in which the sensitivity and specificity have remained the same in Table 17–16, but the prevalence of disease has decreased 10-fold, to 3.3%.

The positive and negative predictive values are now:

$$\text{Positive predictive value} = \frac{54}{334} = 0.16$$

$$\text{Negative predictive value} = \frac{1586}{1596} = 0.99$$

Note that while the negative predictive value has increased slightly, there has been a substantial drop in the positive predictive value. The latter effect is due to the presence of a large number of false positives. Whereas in the population studied in Table 17–16 we could say that a positive test indicated disease in 74% of cases, in the population studied in Table 17–17 a positive test is associated with disease in only 16% of cases.

Note that, as discussed here, predictive value theory applies only to dichotomous tests, ie, tests that are classified as normal or abnormal. In the case of the CSF index, this required the selection of some diagnostic cutoff for the test results that separates normal from abnormal values. The diagnostic sensitivity and specificity change as the cutoff is changed. One way of determining what value should be used for this cutoff is to compute sensitivity and specificity for various values of the cutoff. These results can be plotted as a **receiver operating characteristics curve (ROC)**, which is a plot of sensitivity versus (1 − specificity).

Decision theory provides more sophisticated tools for the analysis of tests reported as values from a continuous scale.

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Clinical Laboratory Methods for Detection of Cellular Immune Function

18

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The immune system in humans can be divided into 2 major parts, one involving humoral immunity (antibody and complement) and the other cellular immunity. In many ways this separation is artificial, and many examples of the interdependence of cellular and humoral immunity could be cited (see Chapter 7). Nevertheless, dividing the immune system into parts in this way has provided a conceptual and practical framework for the laboratory evaluation of immunity in clinical practice.

In the preceding chapter we reviewed methods of detecting antibodies and methods that employ antibodies for antigen detection. The role of a variety of distinct cell types (see Chapter 7) in immune mechanisms in normal and diseased persons has become measurable in the clinical laboratory. Immunocompetent cells, including lymphocytes, monocytes-macrophages, and granulocytes, are all involved in the delayed hypersensitivity reactions that are so important in immunity to intracellular infection, tumor immunity, and transplant rejection. The clinical laboratory investigation of the number and function of these cells is still beset by difficulties in test standardization, biologic variability, the imprecise nature of many assays, and the complexity and expense of the procedures. Nevertheless, several tests that are of value in assessing cellular function have become available for clinical use. Many of these assays employ sophisticated immunochemical methods for detecting cellular antigens or markers. Of great importance is the advent of monoclonal antibodies to detect various leukocyte subsets. Molecular biologic techniques such as Southern blots have recently been employed to detect either immunoglobulin gene or T cell receptor gene rearrangements as markers of specific B and T cell lineages. Thus, we are witnessing an increasing fusion of biochemistry with cellular immunology.

The present chapter reviews the tests that have medical application in the detection of cell types and their corresponding functions. The intention is not to provide a comprehensive laboratory manual but to familiarize the reader with the principles, applications, and interpretation of these assays. Our understanding of cellular immunity continues to expand, and technologic advances in methods for its assessment have been developed.

The topics discussed include (1) delayed hypersensitivity skin tests, (2) assays for T and B lymphocytes, (3) lymphocyte activation, (4) monocyte-macrophage assays, and (5) neutrophil function.

DELAYED HYPERSENSITIVITY SKIN TESTS

Despite the development of a multitude of complex procedures for the assessment of cellular immunity, the relatively simple intradermal test remains a useful tool, occasionally serving to establish a diagnosis. Delayed hypersensitivity skin testing detects cutaneous hypersensitivity to an antigen or group of antigens and, when one is testing for an infectious disease, does not necessarily imply active infection with the agent being tested for. Delayed hypersensitivity skin tests are also of great value in the overall assessment of immunocompetence and in epidemiologic surveys. Inability to react to a battery of common skin antigens is termed **anergy**, and clinical conditions associated with this hyporeactive state are listed in Table 18–1.

Technique of Skin Testing

(1) Lyophilized antigens should be stored sterile at 4 °C, protected from light, and reconstituted shortly before use. The manufacturer's expiration date should be observed.

(2) Test solutions should not be stored in syringes for prolonged periods before use.

(3) A 25- or 27-gauge needle usually ensures intradermal rather than subcutaneous administration of antigen. Subcutaneous injection leads to dilution of the antigen in tissues and can lead to a resultant false-negative test.

(4) The largest dimensions of both erythema and induration should be measured with a ruler and recorded at both 24 and 48 hours.

(5) Hyporeactivity to any given antigen or group of antigens should be confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with the intermediate dose.

Contact Sensitivity

Direct application to the skin of chemically reactive compounds results in systemic sensitization to various

Table 18–1. Clinical conditions associated with anergy.*

- **I. Technical errors in skin testing**
  - Improper dilutions
  - Bacterial contamination
  - Exposure to heat or light
  - Adsorption of antigen on container walls
  - Faulty injection (too deep, leaking)
  - Improper reading of reaction
- **II. Immunologic deficiency**
  - Congenital
    - Combined deficiencies of cellular and humoral immunity
      - Ataxia-telangiectasia
      - Nezelof's syndrome
      - Severe combined immunodeficiency
      - Wiskott-Aldrich syndrome
    - Cellular immunodeficiency
      - Thymic and parathyroid aplasia (DiGeorge syndrome)
      - Mucocutaneous candidiasis
  - Acquired
    - AIDS
    - Sarcoidosis
    - Chronic lymphocytic leukemia
    - Carcinoma
    - Immunosuppressive medication
    - Rheumatoid diseases
    - Uremia
    - Alcoholic cirrhosis
    - Biliary cirrhosis
    - Surgery
    - Hodgkin's disease and lymphomas
- **III. Infections**
  - Influenza
  - Mumps
  - Measles
  - Viral vaccines
  - Typhus
  - Miliary and active tuberculosis
  - Disseminated mycotic infection
  - Lepromatous leprosy
  - Scarlet fever

*Modified from Heiss LI, Palmer DL: Anergy in patients with leukocytosis. *Am J Med* 1974;56:323.

metabolites of the sensitizing compound. The precise chemical fate of the sensitizing compound is not known, but sensitizing agents such as dinitrochlorobenzene (DNCB) probably form dinitrophenylprotein complexes with various skin proteins. Sensitization with DNCB can be used in skin testing for delayed hypersensitivity in a few selected patients with suspected anergy. It is not a routine procedure and should be reserved for instances in which thorough delayed hypersensitivity testing with other antigens is negative. Furthermore, its use as a diagnostic reagent is not currently approved by the FDA. Following application of DNCB to the skin, a period of about 7–10 days elapses before contact sensitivity can be elicited by a challenge dose applied to the skin surface. This sensitivity persists for years. The ability of a subject to develop contact sensitivity is a measure of cellular immunity to a new antigen to which the subject has not been previously exposed. Thus, the establishment of a state of cutaneous anergy in various disease states may be confirmed and extended by testing with DNCB.

Interpretation of contact sensitivity reactions depends on development of a flare or vesicular reaction at the site of challenge. Induration rarely occurs, since the test dose is not applied intradermally. In some clinical situations, a nonspecific depression in the inflammatory response can result in apparent anergy. A local irritant such as croton oil is occasionally employed to test the ability to mount a nonspecific inflammatory response.

Patch testing is commonly employed by allergists and dermatologists to detect cutaneous hypersensitivity to various substances thought to be responsible for contact dermatitis. The test substance is applied in a low concentration and the area covered with an occlusive dressing. After 24–48 hours, the dressing is removed and the site examined for presence of a superficial inflammatory reaction. False-positive reactions can result from too high a concentration of the test substance, irritation rather than allergy, and allergy to the adhesive in the dressing. False-negative tests usually result from too low a concentration of the test substance or inadequate skin penetration. The results of patch testing must be carefully weighed with the clinical history and knowledge of the chemistry of the potential sensitizing agent. (See also Chapters 24 and 29.)

Interpretation & Pitfalls

The inflammatory infiltrate that occurs 24–48 hours following intradermal injection of an antigen consists primarily of mononuclear cells. This cellular infiltrate and the accompanying edema result in induration of the skin, and the diameter of this reaction is an index of cutaneous hypersensitivity. A patient may also demonstrate immediate hypersensitivity to the same test antigen, ie, a coexistent area of erythema (wheal and flare) which usually fades by 12–18 hours but may occasionally persist longer (see Chapter 15). Induration of 5 mm or more in diameter is the generally accepted criterion of a positive delayed skin test. Smaller but definitely indurated reactions suggest sensitivity to a closely related or cross-reacting antigen. There is no definitive evidence that repeated skin testing can result in conversion of delayed hypersensitivity skin tests from negative to positive. However, with some antigens, intradermal testing can result in elevations of serum antibody titers and confuse a serologic diagnosis. For this reason, blood for serologic study should always be obtained before skin tests are performed.

Delayed hypersensitivity skin testing is of relatively little value in establishing the diagnosis of defective cellular immunity during the first year of life. Infants may fail to react owing to lack of antigen contact, with resultant sensitization to various test antigens. Consequently, in vitro assay for T cell numbers and function is much more useful in the diagnosis of congenital immunodeficiency disease (see Chapter 20). Recent evidence has also associated failure to re-

spond to various tuberculin antigens to genetic markers of HLA-DR.

In late 1977, an FDA panel submitted a highly critical report to the Commissioner of the FDA on the safety and efficacy of several microbial skin test antigens. The evaluation considered various manufacturers' preparations of histoplasmin, tuberculin (PPD), coccidioidin, *Trichinella* extract, old tuberculin, diphtheria toxin for Schick test, lymphogranuloma venereum antigen, and mumps skin test antigen. The Panel on Review of Skin Test Antigens found only 5 of the 23 tested products to be safe and effective. Immediate removal of 12 and temporary licensing of the remaining 6 were implemented by the Commissioner. The details of this report are published in *Federal Register* 1977;**42(Sept 30)**:52, 674, which is published by the US Government Printing Office, Washington, DC.

Possible Adverse Reactions to Skin Tests

Occasional patients who are highly sensitive to various antigens will have marked local reactions to skin tests. These are most likely to occur with second strength and more concentrated doses. Reactions include erythema, marked induration, and, rarely, local necrosis. Patch testing may rarely result in sensitization. Systemic side effects such as fever or anaphylaxis are uncommon. Injection of corticosteroids locally into hyperreactive indurated areas may modify the severity of the reaction. Similarly, the painful blistering and inflammation that sometimes occur following surface application of DNCB can be reduced by topical corticosteroids.

ASSAYS FOR HUMAN LYMPHOCYTES & MONOCYTES

The era of modern cellular immunology began with the discovery that lymphocytes are divided into 2 major functionally distinct populations. Evidence for the existence of T (thymus-derived) and B (bursa-derived) lymphocytes in humans originated from studies of other mammalian and avian species and analysis of lymphocyte populations in immunodeficiency diseases (see Chapter 13). Extensive studies of cell surface markers with monoclonal antibodies and in vitro functional assays have provided direct evidence for the existence of these major lymphocyte types and several others. Prominent among these are natural killer (NK) cells.

The terms T lymphocyte and B lymphocyte usually denote the functional entities of the 2 major classes of immunocompetent cells in peripheral blood. T lymphocytes arise in the thymus and migrate to peripheral lymphoid organs—lymph nodes, spleen, and blood—during embryonic life. B lymphocytes mature during embryogenesis by a functional—but as yet anatomically undefined—equivalent of the avian bursa of Fabricius. T lymphocytes function as effector cells in cellular immune reactions, cooperate with B cells to form antibody (helper function), and suppress certain B cell functions (suppressor function). After appropriate antigenic stimulation, B lymphocytes differentiate into plasma cells that eventually secrete antibody. The notion that there is only one type of T cell or B cell has been found to be an oversimplification, since subclasses of T and B cells are now recognized. Furthermore, definitive assignment of lymphocytes to one or the other of these classes or subclasses is not always possible.

Assays for T and B cells are currently in wide use in clinical immunology. Enumeration of leukocytes is performed by microscopy or flow cytometry using specific membrane antibodies linked either to fluorescent dyes or to enzymes that produce reactants. Such techniques can be applied either in tissue sections or in fresh suspensions of cells from blood, bone marrow, or other organs. Precise counting of T and B cells in human peripheral blood has made important contributions to our understanding of (1) immunodeficiency disorders, (2) autoimmune diseases, (3) tumor immunity, and (4) infectious disease immunity. It should be emphasized, however, that mere counting of T or B cells does not necessarily correlate with the functional capacity of these cells. At best, these assays provide a nosologic classification of immunocompetent cells; further evaluation of lymphocyte function usually should be performed to fully assess immunologic competence in clinical practice.

In 1983, the First International Workshop on Human Leukocyte Differentiation Antigens met and established a new nomenclature for immunologically defined cellular types and subtypes. They defined a series of "clusters of differentiation" (CD) types that define cellular antigens. In 1984, the second workshop refined and expanded this nomenclature. Definition of these CD types and relationship to other antigen or antibody designations are presented in Table 18–2. It should be emphasized that the CD nomenclature is rapidly replacing the more familiar, often proprietary, antibody designations, eg, OKT 4, Leu 3, etc.

Separation of Peripheral Blood Mononuclear Cells for Lymphocyte & Monocyte Assays

Tests for human T and B cells are ordinarily performed on purified suspensions of mononuclear blood cells. An accepted procedure for obtaining cell suspensions is density gradient centrifugation on Ficoll-Hypaque. This method results in a yield of 70–90% mononuclear cells with a high degree of purity but may selectively eliminate some lymphocyte subpopulations. Mononuclear preparations obtained by this method are relatively enriched in *monocytes*. These cells must be distinguished from lymphocytes by morphologic characteristics, phagocytic ability, endogenous enzymatic activity, or cell surface antigens (see below).

In order to avoid misinterpretation, results of tests

Table 18–2. T cell differentiation antigens.

Cell Type Marked	CD Designation	Antibody Designation	Comments
Cortical thymocytes	CD1	Leu 6 OKT 6	Early T cell antigen also present on Langerhans cells, associated with β_2-microglobulin not present on peripheral T cells.
E rosette-forming cells	CD2	Leu 5 OKT 11 T11	Pan-T cell antigen SRBC receptor on T cells.
Mature T cells	CD3	Leu 4	Also present on T cell ALL and cutaneous T cell lymphoma.
Helper/inducer T cells	CD4	Leu 3 OKT 4 T4	Can be further subdivided into helper and inducer subsets.
Pan-T and -B cell subpopulation	CD5	Leu 1 T1 T101 OKT 1	B cell CLL. B cells following marrow transplant.
Mature T and B cell subpopulation	CD6	T12	Malignant T cells.
Pan-T cells, thymocytes	CD7	Leu 9 3A1	T cell leukemias.
Suppressor/cytotoxic T cells	CD8	Leu 2 OHT 8 T8	Can be further subdivided into cytotoxic and suppressor subsets.

for T and B cell markers on separated populations should generally be expressed as the number of cells per microliter of whole blood. Many published studies have indicated only the percentages of lymphocytes carrying a particular marker. Such a result could be due to an increase in the particular cell population or, alternatively, a decrease in other populations. Thus, it is important that each laboratory establish standard absolute numbers of T and B cells per microliter of whole blood from normal individuals.

Methods for assessing cellular phenotypes utilizing whole unseparated blood have been developed. Cells are stained with fluorochrome-conjugated monoclonal antibodies, and red cells are lysed. The residual washed leukocytes are counted by flow cytometry or fluorescence microscopy. The whole blood method has the advantage of simplicity but has not yet been fully evaluated in many diseases. It is generally not useful for functional studies owing to potential interference by red blood cells or plasma proteins.

T LYMPHOCYTE ASSAYS

Human T Cell Antigens

Production of heteroantisera to normal and malignant human T cells created the potential for direct immunochemical detection of cellular subpopulations by immunofluorescence and other sensitive techniques. However, with few exceptions, conventional antisera raised in animals to T cell subsets have lacked sufficient specificity owing to extensive cross-reactivity and broad response to species-specific rather than lineage-specific antigens on the immunizing cells. Some degree of improvement in the quality of these reagents was achieved by use of naturally occurring human antibody derived from sera of patients with various autoimmune diseases or by use of purified or continuously cultured T cell subpopulations. However, with the advent of monoclonal antibodies produced by murine hybridomas (see Chapter 17), a major breakthrough has been achieved in identification of human T cells.

Monoclonal antibodies have been produced in many laboratories to class-specific and subclass-specific T cell antigens. These antibodies are highly specific and provide sensitive reagents for detecting cells in suspensions or fixed tissue sections. An enormous proliferation of abbreviations for these sera has occurred simultaneously with their commercial availability. The use of CD terminology, which is compared to more common proprietary designations, is found in Table 18–2. It is anticipated that new antigens defining specialized subsets of T cells will continue to emerge for the current groupings.

A. Performance of Test:

1. Production of T cell antibodies—T cells from various sources—especially thymocytes, purified peripheral blood T cells, T leukemia cells, or T cells from continuous culture—can be used for immunization of either goats or rabbits. The resulting antisera have both species-specific and T cell-specific antibodies. The former may be removed by absorption with B cells, liver, or kidney cells. Specificity must be shown by positive reaction with E rosette-forming cells and negative reaction with B cells. Monoclonal antibody is produced from murine hybridomas (see Chapter 17).

2. Detection of T cell markers with specific antisera—Immunofluorescence of either live lymphocytes or frozen tissue sections is possible. Direct

immunofluorescence is performed with fluorochrome-labeled γ-globulin fractions from anti-T cell serum or labeled hybridoma culture supernates.

In vitro cytotoxicity of human T cells by specific antisera may also be used to estimate T cell populations. Methods for assessing T cell killing include trypan blue vital staining or ^{51}Cr release assay.

B. Interpretation: The percentage or absolute number of T cells or T cell subsets is determined by their binding to various specific antibodies. Cells are counted by direct observation using a fluorescence microscope or by flow cytometer (see below). The latter analysis is beginning to replace microscopy in clinical laboratories as simpler and less expensive instruments are developed. The overwhelming advantages of objectivity, sensitivity, and speed make flow cytometric analysis preferable to the tedious process of counting cells by microscopic observation.

T Cell Subsets

Major subsets of T cells consist of helper and suppressor types. However, helper cells (CD4) consist of at least 2 phenotypically and functionally distinct subtypes: inducer cells ($CD4^+$, Leu 8^+, or $TQ1^+$), which influence induction of mature helper cells and suppressor cells; and helper cells ($CD4^+$, Leu 8^-, or $TQ1^-$), which influence antibody production of B cells. These 2 subtypes of the CD4 class are recognized phenotypically by the simultaneous expression of CD4 molecules and either Leu 8 or TQ1 (which have not yet received CD designations). So far, no single reagent has been developed that can identify these populations.

Suppressor cells (CD8) can similarly be subdivided into so-called true suppressor cells ($CD8^+$, $CD11^+$), which influence B cell antibody function, and cytotoxic T cells ($CD8^+$, $CD11^-$). Combinations of monoclonal antibodies are thus also used to detect these 2 important T cell subsets.

Helper:Suppressor Cell Ratios (H/S Ratio)

Largely because of the interest and concern generated by the current AIDS (acquired immunodeficiency syndrome) epidemic, many laboratories express results of helper T/inducer and suppressor T/cytotoxic counts as a ratio or quotient. Caution must be exercised in using this approach, since the ratio may vary depending on changes in either numerator or denominator or both. Also, standardization of normal values and the clinical significance of slight deviations from the normal range (~ 1.8–2.2) are not well understood. Diseases or conditions that have been reported to be associated with high or low helper:suppressor ratios are presented in Table 18–3. Obviously, this laboratory test is not diagnostic of any particular condition and has to be interpreted cautiously based on the persistence or transience of the abnormality. In AIDS, for example, the reduction in the ratio seems to be permanent, whereas in some viral infections, eg, cytomegalovirus, it is reversible.

Table 18–3. Helper:suppressor cell ratios in human peripheral blood.

Decreased in	Increased in
SLE with renal disease	Rheumatoid arthritis
Acute cytomegalovirus infection	Type I insulin-dependent diabetes mellitus
Burns	SLE without renal disease
GVH disease	Primary biliary cirrhosis
Sunburn or ultraviolet solarium exposure	Atopic dermatitis
Myelodysplasia syndromes	Sézary syndrome
Acute lymphocytic leukemia in remission	Psoriasis
Recovery from bone marrow transplant	Chronic autoimmune hepatitis
AIDS	
Herpes infections	
Infectious mononucleosis	
Measles	
Vigorous exercise	

E Rosette-Forming Cells

Human T cells were formerly identified by their ability to bind sheep red blood cells (SRBC) to form rosettes (Fig 18–1). However, detection of this property has largely been replaced by more sensitive binding of monoclonal antibodies that identify the SRBC receptor. This receptor is now designated CD2. The E rosette test occasionally is used to compare the morphology of lymphocytes with their ability to express this receptor in lymphoid cancer.

B LYMPHOCYTE ASSAYS

Surface Immunoglobulin

Lymphocytes with readily demonstrable surface immunoglobulin are B cells. This surface immunoglobulin is synthesized by the lymphocyte and under ordinary conditions does not originate from serum; ie, it is not cytophilic antibody. Lymphocytes generally bear monoclonal surface immunoglobulin, ie, immunoglobulin of a single H chain class and L chain type.

A. Performance of Test: Polyspecific antisera against all immunoglobulin classes permit detection of total numbers of B cells in a blood sample. Alternatively, a mixture of anti-κ and anti-λ antisera will detect total numbers of B cells. Monospecific antisera are developed by immunization with purified paraproteins and appropriate absorptions.

Tests for surface immunoglobulin-bearing B cells are performed by direct immunofluorescence with fluorochrome-labeled γ-globulin fractions derived from heterologous anti-immunoglobulin antisera. A major difficulty is to ensure the absence of all aggregated immunoglobulin in these test reagents (see below).

Generally, small amounts of anti-immunoglobulin antisera are mixed with purified lymphocyte suspensions for 20–30 minutes at 4 °C. After removal of unbound immunoglobulin, the presence of surface im-

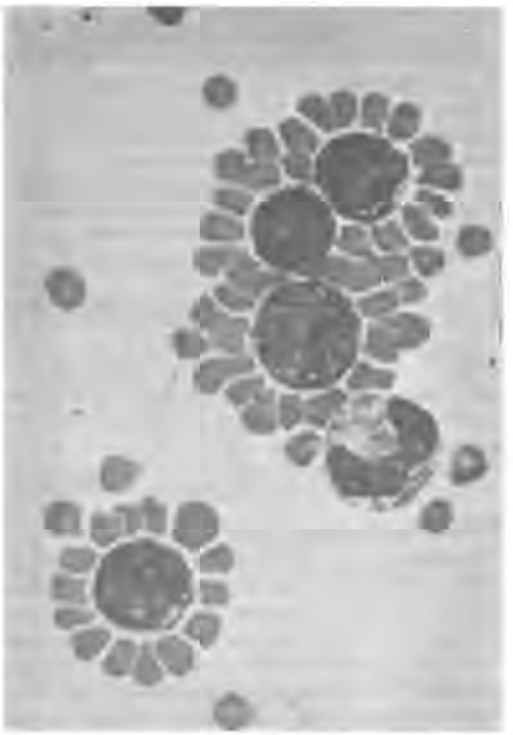

Figure 18–1. E rosette-forming cells. Lymphocytes from human peripheral blood that have formed rosettes with sheep erythrocytes. Such cells are T lymphocytes. A granulocyte has failed to form a rosette. (Courtesy of M Kadin.)

munoglobulin is determined by counting in a fluorescence microscope.

Table 18–4 summarizes data on the numbers of surface immunoglobulin-bearing B cells in normal subjects. In certain disease states, eg, systemic lupus erythematosus, antilymphocyte antibody may be bound to B or T cells in vivo. In order to prove that surface immunoglobulin is a metabolic product of that cell, enzymatic removal and resynthesis of surface immunoglobulin may be performed in vitro.

B. Interpretation: The percentages of IgG-bearing lymphocytes are in fact considerably lower than previously reported. Falsely high levels are detected owing to formation of IgG-anti-IgG complexes at the cell surface with bonding to B cells via the Fc receptor. When $F(ab)'_2$ anti-IgG reagents were prepared, the percentage of IgG-bearing cells was reduced from 5% to less than 1%.

The problem of binding of anti-immunoglobulin reagents to Fc receptors on B cells and monocytes is particularly a problem with rabbit anti-human antisera. A recent study suggests that nonspecific binding is almost eliminated by use of goat or sheep antisera to human immunoglobulins. Most investigators agree that IgM and IgD are the predominant surface immunoglobulins on human peripheral B lymphocytes.

Table 18–4. Surface immunoglobulin-bearing B lymphocytes in normal adult blood.*

Surface Immunoglobulin	Mean %	Range
Total Ig	21	16–28
IgG	7.1	4–12.7
IgA	2.2	1–4.3
IgM	8.9	6.7–13
IgD†	6.2	5.2–8.2
IgE‡	...	...
κ	13.9	10–18.6
λ	6.8	5–9.3

*From: WHO Workshop on Human T & B Cells. *Scand J Immunol* 1974;3:525.

†IgD and IgM are frequently expressed on the same cell.

‡IgE B cells are extremely rare.

Cytoplasmic Immunoglobulins

In some lymphoid malignancies, particularly Waldenström's macroglobulinemia, chronic lymphocyte leukemia, or B cell lymphomas with leukemia, circulating lymphocytes with monoclonal intracytoplasmic immunoglobulins are detected. This immunoglobulin is usually identical to the molecule found on the surface of these cells and is occasionally present as a paraprotein in serum. Rarely, the intracellular immunoglobulin forms distinct crystals that appear as spindles or spicules within cytoplasm.

Recently, a group of patients with acute lymphocytic leukemias have been described with pre-B cells that express only intracytoplasmic IgM and no surface immunoglobulins at all. It is important to test for intracellular IgM particularly in so-called null cell acute lymphocytic leukemia patients, since the group with pre-B cell leukemia is probably a distinct clinical subgroup with a different course and prognosis.

Detection of intracellular immunoglobulins is done by direct immunofluorescence with specific anti-heavy chain or light chain sera on acetone- or ethanol-fixed cytocentrifuged preparations of purified lymphocytes.

EAC (Erythrocyte-Amboceptor-Complement) Rosettes

A certain percentage of lymphocytes contain surface receptors for complement. This B cell marker is most readily demonstrated by a rosetting technique similar to E rosette assay. However, to avoid confusion with T cell rosettes, ox cells that do not bind spontaneously to human lymphocytes may be used. Ox red blood cells are coated with IgM antibody to red blood cells in the presence of complement deficient in C5 to avoid red cell lysis. These C- and antibody-coated erythrocytes are called EAC. The EAC are then incubated with separated lymphocytes, and rosetted cells surrounded by 3 or more EAC cells are considered positive. Fresh serum-treated yeast cells or zymosan particles can also be used to detect C3 receptors on B cells.

The mean value for EAC rosette-forming cells from various laboratories is 15.4%, with a reported

range of 10–19%. Since monocytes and polymorphonuclear leukocytes may also form EAC rosettes, other procedures are necessary to identify these cells. However, this test has the advantage of not requiring fluorescence microscopy.

Anti-B Cell Antibody

Purified B cells can be used to develop antisera for B cell detection. Large numbers of homogeneous B cells can be obtained from the blood of patients with chronic lymphocytic leukemia. Naturally occurring antibodies from pregnant women or multiply transfused patients can react with Ia-like determinants or B cells. These antisera are useful in detecting HLA-D antigens and as class-specific reagents for B cell detection. Monoclonal antibodies to Ia antigen (HLA-DR) have been produced and are useful in detecting B cells. Additional monoclonal antibodies to detect lineage-specific B cell antigen have been developed that detect B cells at various developmental stages.

Functional B Cell Assays

In the clinical laboratory, B cell function has been traditionally measured by the assessment of immunoglobulin levels or antibody titers, since these are the end products of B cell differentiation. However, 2 additional in vitro approaches to assessing functional abnormalities in B cells are now available. These are B cell activation by mitogens and immunoglobulin synthesis and secretion.

A. B Cell Activation by Mitogens: B cells can be stimulated to proliferate by several mitogens. Pokeweed mitogen functions with T cell cooperation and is not a direct B cell test. However, staphylococcal protein A (Cowan I strain) (SAC) directly stimulates B cell activation. Measurement of this B cell attribute is analogous to PHA or Con A stimulation previously described for T cells.

B. Immunoglobulin Biosynthesis: B cell activation by antigens or mitogens results in small but detectable quantities of polyclonal immunoglobulins. Following 7–10 days of culture, these products are measured by RIA or ELISA methods. Alternatively, B cells that produce immunoglobulins can be quantified by the reversed hemolytic plaque assay. In this assay, erythrocytes are coated with goat or rabbit anti-human immunoglobulins. They are mixed with putative immunoglobulin-producing lymphocytes and semisolid agar, and complement is added. The presence of hemolytic plaques indicates the presence of immunoglobulin-producing cells.

B cells that have differentiated into plasma cells during an in vitro assay can be enumerated by staining for intracellular immunoglobulins by direct immunofluorescence in fixed smears of cultured cells.

The advantage of these in vitro B cell function tests is that they allow for delineation of immunoregulatory defects involving T or B cells by substitution of various cell populations among healthy and diseased cell donors. Although not yet routinely available, specific antibody assays for direct in vitro study are becoming available. This exciting prospect may allow new insights into the pathogenesis of many immune disorders.

LEUKEMIA CELL-ASSOCIATED ANTIGENS

Terminal Deoxynucleotidyl Transferase (TdT)

Terminal deoxynucleotidyl transferase (TdT) is an enzyme that catalyzes the polymerization of deoxynucleoside triphosphates in the absence of a template. The enzyme is a marker for immature cells in the hematopoietic system. It is present in approximately 90% of cortical thymocytes and about 2% of bone marrow cells. Although transiently present in blood and other lymphoid organs during embryogenesis, it is not present in normal tissues (except marrow and thymus) in adult life.

TdT is a useful clinical marker for the presence of immature T cells in patients with leukemias and lymphomas. It can be detected by either enzyme assay or cellular homogenates or in fixed smears by immunofluorescence. There are increased numbers of TdT-containing cells in the marrow of nearly all patients with acute lymphocytic leukemia and in many cases of T cell lymphoma. Frequently the marker is present in the leukemic population that occurs in blast crisis of chronic myelogenous leukemia. It is not present in mature peripheral T cells and thus can best be considered a marker for pre-T cells and possibly other immature hematopoietic cells.

Common Acute Lymphoblastic Leukemia Antigen (CALLA)

This antigen, to which both monoclonal and conventional antisera have been prepared, is found on the tumor cells in approximately 80% of patients with acute lymphoblastic leukemia and 40–50% of patients with chronic myelogenous leukemia in blast crisis. It has a molecular weight of 100,000 and is detected by the monoclonal antibodies J5, J13, and 24.1. Originally thought to be a tumor-specific antigen for acute lymphocytic leukemia, the antigen is also expressed on other cells including 2–6% of normal nucleated bone marrow cells, fibroblasts in tissue culture, and several nonhematopoietic tumor cell lines. It has approximately the same clinical value as terminal transferase (TdT), since most large series show striking concordance between presence of CALLA and TdT in many leukemias.

FLOW CYTOMETRY & CELL SORTING

Biochemical and biophysical measurements of single cells have been performed for years, primarily using visual analysis in various types of microscopes. Many of the immunohistochemical methods described

in Chapter 17—and the cellular analysis methods discussed above—have become increasingly refined through the development of a new class of instruments called flow cytometers. A detailed description of the myriad applications of this general technique is beyond the scope of our discussion. In brief, flow cytometers are instruments capable of analyzing properties of single cells as they pass through an orifice at high velocity. Examples of measurements that can be made include physical characteristics such as size, volume, refractive index, and viscosity and chemical features such as content of DNA and RNA, proteins, and enzymes. These properties are detected by measuring light scatter, Coulter volume, and fluorescence. Instruments have been designed solely to analyze these properties and are combined with sophisticated electronics and computers. However, another class of even more sophisticated instruments—cell sorters—combine analytic capacity with the ability to sort cells based on various preselected properties. One type of sorter, the fluorescence-activated cell sorter (FACS), has found many applications in immunologic research. Since this type of machine is gradually being introduced into clinical laboratory immunology, particularly for analysis of T, B, and other lymphoid cells, a description of the principles of its operation is presented here.

Cell Analysis by Flow Cytometry

Counting individual cells in complex mixtures is a tedious and imprecise technique even with monoclonal fluorescent antibodies and sophisticated microscopes. With the aid of a flow cytometer used as an analytic instrument, a single cell suspension may be analyzed for various measurements simultaneously at the rate of nearly 5000 cells per second. By combining light scatter and Coulter volume measurements with the powerful tool of fluorescently labeled monoclonal antibodies, subpopulations can be easily identified. A typical histogram produced by analysis of human T cells is shown in Fig 18–2. The number of cells under the curves can be determined and thereby the percentage of positive and negative cells in relation to an arbitrary threshold of fluorescent signal.

The use of 2 differently colored fluorochromes each coupled to a particular antibody allows simultaneous 2-color immunofluorescence of individual cells. Recently, flow cytometers have been developed which can excite 2 different dyes that absorb light at similar wavelengths but emit light in red and green. A "dot plot" graph of the results of simultaneous 2-color immunofluorescence in the analysis of activated DR antigen-positive suppressor cells in AIDS is shown in Fig 18–3. The immense power of these refined techniques is just beginning to be explored in clinical laboratories.

Cells larger and smaller than lymphocytes can be "gated out" electronically so that the analysis concentrates solely on lymphocytes. With the addition of 90-degree-angle light scatter, granulocytes can also be identified and "gated out." This approach has allowed

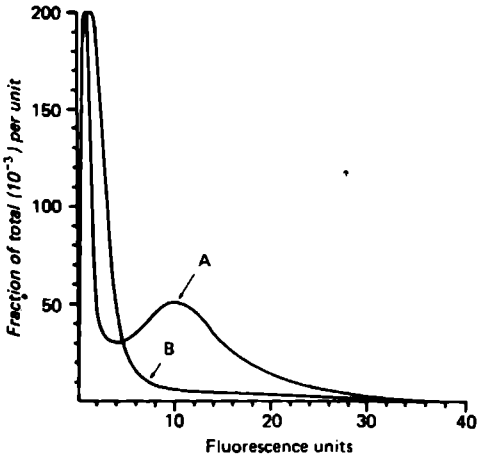

Figure 18–2. Single-color immunofluorescence histogram from flow cytometric analysis of human T cells. Human lymphocytes were stained with fluorescently labeled anti-T cell antibody *(A)* and control nonreactive FITC-labeled antiserum *(B)*. In both patterns, a high, sharp peak of autofluorescence from unstained cells is observed near the y-axis. However, in the curve labeled *(A)* stained with anti-T cell antibody, a significant peak appears at about 11 fluorescence units. No such increase in the number or intensity of fluorescent cells is observed in the control *(B)* peak. (Modified and reproduced, with permission, from: Melamed MR, Mullaney PF, Mendelsohn ML: *Flow Cytometry and Sorting*. Wiley, 1979.)

the development of whole blood methods for lymphoid cell analysis. Further refinement in this method will make these techniques more widely applicable in clinical laboratories.

Fluorescence-Activated Cell Sorters

A single cell suspension is isolated from blood or other tissues and labeled with either fluorescent antibody or another fluorochrome dye such as ethidium bromide, which specifically stains DNA (Fig 18–4). The cells are forced under pressure through a nozzle in a liquid jet surrounded by a sheath of saline or water. Vibration at the tip of the nozzle assembly causes the stream to break up into a series of droplets, and the size of the droplets can be regulated so that each will contain exactly one cell. The droplets are illuminated by the monochromatic laser beam and electronically monitored by fluorescence detectors. Droplets that emit appropriate fluorescent signals are electrically charged in a high-voltage field between deflection plates and are then sorted into collection tubes. Rapid, accurate, and highly reproducible separation of cells is thereby accomplished. Viability and sterility can be maintained, so that cells can be not only analyzed but also cultured or assayed functionally.

Clinical Applications of Flow Cytometry

The flow cytometer has had many applications in immunology. A partial list would include the follow-

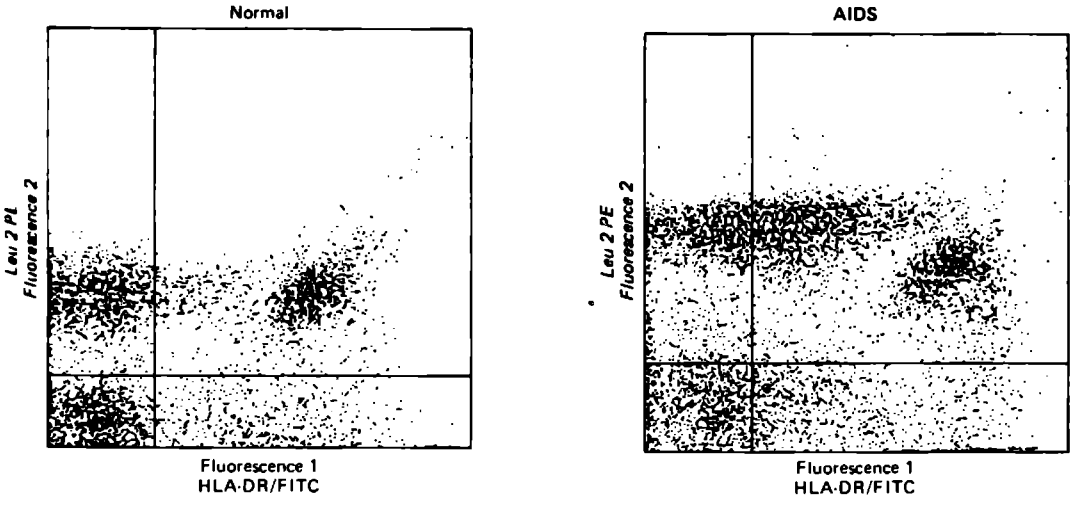

Figure 18–3. Effect of DR antigen on suppressor cells. Simultaneous 2-color immunofluorescence analysis of human suppressor T cells also bearing DR antigens. Human lymphocytes were stained with an FITC-labeled anti-DR antibody and a phycoerythrin-labeled anti-suppressor T cell antibody. The dot plot represents individual cells, with FITC-labeled cells on the x-axis and phycoerythrin-labeled cells on the y-axis. The coordinate of an individual dot thus indicates whether it is labeled with one or both antibodies. In addition, monocytes are labeled with antisera containing both fluorochromes and appear in both panels in the upper right quadrant. There is an increase in the number of doubly stained cells in AIDS versus the control panel.

ing: (1) analysis and sorting of subpopulations of T cells by monoclonal fluorescent antibodies; (2) separation of various classes of lymphoid cells through sorting by size or antibody marker; (3) separation of live from dead cells; (4) cloning of individual cells by introducing microtiter plates in place of collection tubes; (5) analysis of cell cycle kinetics by various DNA stains; and (6) detection of rare cells such as monoclonal B cells in the blood of lymphoma patients. Computers are used to analyze multiple parameters measured simultaneously by the flow cytometer, including 2-color fluorescence, forward-angle light scatter, and 90-degree-angle light scatter. It is anticipated that many of the assays described for T and B cells will soon be performed in clinical laboratories with the aid of microfluorimetry in flow cytometers.

LYMPHOCYTE ACTIVATION

Lymphocyte activation or stimulation refers to an in vitro correlate of an in vivo process that regularly occurs when antigen interacts with specifically sensitized lymphocytes in the host (see Chapters 7 and 8). Lymphocyte transformation is a nearly synonymous term first used by Nowell in 1960 and later by Hirschhorn and others to describe the morphologic changes that resulted when small, resting lymphocytes were transformed into lymphoblasts on exposure to the mitogen phytohemagglutinin (PHA). Blastogenesis refers to the process of formation of large pyroninophilic blastlike cells in cultures of lymphocytes stimulated by either nonspecific mitogens or antigens.

Lymphocyte activation is an in vitro technique commonly used to assess cellular immunity in patients with immunodeficiency, autoimmunity, infectious diseases, and cancer. A myriad of complex biochemical events occurs following incubation with mitogens. These are substances that stimulate large numbers of lymphocytes and do not require a sensitized host, as is the case with antigens. These biochemical events include early membrane-related phenomena such as increased synthesis of phospholipids, increased permeability to divalent cations, activation of adenylate cyclase, and resultant elevation of intracellular cAMP. Synthesis of protein, RNA, and finally DNA occurs shortly thereafter. It is this latter phenomenon, the increase in DNA synthesis, that eventually results in cell division and is the basis for most clinically relevant assays for lymphocyte activation. Convenience and custom have led clinical immunologists to use DNA synthesis rather than earlier events, eg, cAMP or phospholipid metabolism, as a marker for lymphocyte activation.

Although the relationship between lymphocyte activation and delayed hypersensitivity is not always absolute, the method has found widespread use in clinical immunology. The in vivo delayed hypersensitivity skin test is actually the result of a series of complex phenomena including antigen recognition, lymphocyte-macrophage interaction, release of soluble lymphocyte mediators, and changes in vascular permeability. In vitro methods such as lymphocyte

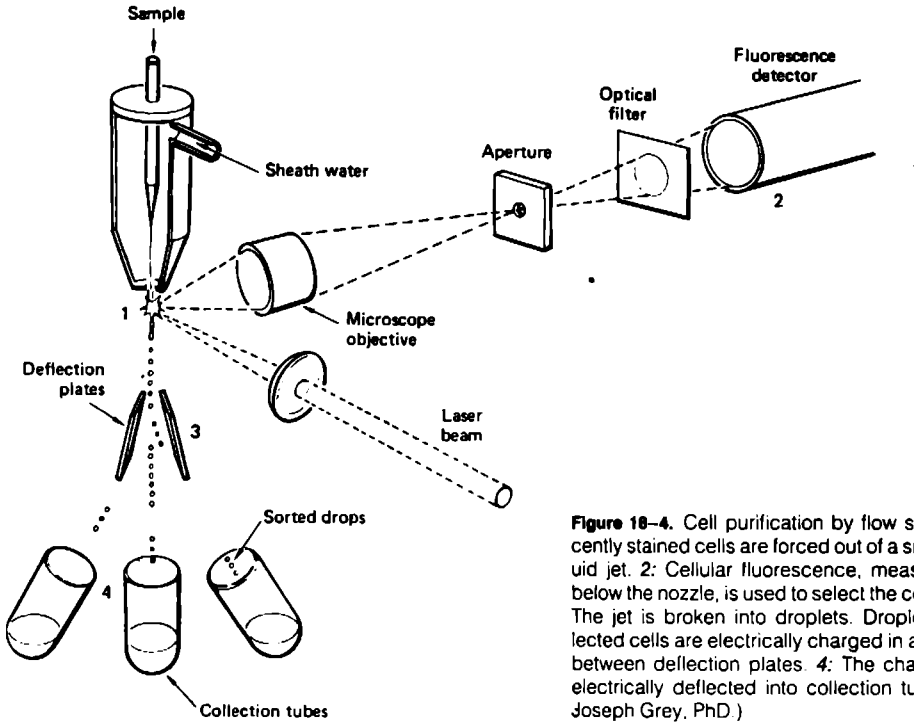

Figure 18–4. Cell purification by flow sorting. *1:* Fluorescently stained cells are forced out of a small nozzle in a liquid jet. *2:* Cellular fluorescence, measured immediately below the nozzle, is used to select the cells to be sorted. *3:* The jet is broken into droplets. Droplets containing selected cells are electrically charged in a high-voltage field between deflection plates. *4:* The charged droplets are electrically deflected into collection tubes. (Courtesy of Joseph Grey, PhD.)

activation are useful for studying cellular hypersensitivity, since they permit analysis of specific stages in the immune response. In addition, they avoid challenge of the patient with potentially detrimental antigens such as drugs, transplantation antigens, or tumor antigens. Lymphocyte activation measures the *functional* capability of T or B lymphocytes to proliferate following antigenic challenge and is therefore a more reliable test of immunocompetence than merely counting types of lymphocytes (ie, T and B cell assays).

Lymphocyte responses can be suppressed or even augmented by a variety of nonspecific factors present in human serum. This humoral modulation of responses to antigens or mitogens should be clearly differentiated from intrinsic suppression of cellular reactivity. Therefore, it is essential to avoid culture of lymphocytes in serum that may contain inhibitory substances. Their presence can usually be excluded by careful questioning of serum donors. If it is suspected that an individual's serum contains an inhibitor of lymphocyte activation, controls should be done utilizing carefully washed cells from that individual cultured in normal serum. A partial list of serum suppressive factors and drugs that may influence in vitro lymphocyte responses is presented in Table 18–5. A note of caution is warranted regarding the significance of this heterogeneous group of substances. Despite clear demonstration of substances with in vitro effects on lymphocyte responses, their in vivo action, particularly in view of the high concentrations often used in tissue culture, remains a matter of speculation.

Table 18–5. Examples of lymphocyte suppressive factors in serum.

Serum proteins	
Albumin (high concentration)	
Specific antibodies to stimulating antigens	
Immunoregulatory globulin	
Alpha-1-acid glycoprotein	
Pregnancy-associated serum globulins	
C-reactive protein (CRP)	
Serum alpha globulin of amyloid (SAA)	
Alpha globulins in cancer, chronic infection, inflammatory diseases	
Alpha-fetoprotein (AFP)	
Low-density lipoprotein	
Antigen-antibody complexes	
HLA antibodies	
T cell antibodies	
Normal serum inhibitors (poorly characterized)	
Hormones	
Glucocorticoids	Androgens
Progesterone	Prostaglandins
Estrogens	
Drugs	
Aspirin	Chloroquine
Cannabis	Ouabain
Others	
Interferon	Chalones
Cyclic nucleotides	

METHODS & INTERPRETATIONS

Mitogen Stimulation

A number of plant lectins and other substances have been employed in assessing human lymphocyte function (Table 18–6). In contrast to studies in mice, there is no incontrovertible evidence that T or B lymphocytes are selectively activated by nonspecific mitogens. PHA and Con A are predominantly T cell mitogens, whereas pokeweed mitogen stimulates B cells. Neither lipopolysaccharide nor anti-immunoglobulin antibody appears to be a potent B cell stimulant in humans. Staphylococcal protein A from *Staphylococcus aureus* cell walls may be a specific stimulant of human B cells, possibly by triggering cells into DNA synthesis via the Fc receptor for IgG.

Lymphocyte Culture Technique for Mitogen Stimulation

Lymphocytes are purified from heparinized peripheral blood by density gradient centrifugation on Ficoll-Hypaque. Cultures are set up in triplicate in test tubes or microtiter trays at a cell concentration of approximately 1×10^6 lymphocytes per milliliter. The culture medium is supplemented with 10 to 20% serum—either autologous, heterologous, or pooled human sera. Mitogens are added in varying concentrations on a weight basis, usually over a 2–3 log range. Cultures are incubated in a mixture of 5% CO_2 in air for 72 hours, at which time most mitogens produce their maximal effect on DNA synthesis. The rate of DNA synthesis was originally estimated by morphologic assessment of the percentage of lymphoblasts present in the culture. However, this method has been largely abandoned owing to the extreme variability and subjectivity of the results. A more accurate measure of DNA synthesis is accomplished by pulse-labeling the cultures with tritiated thymidine (^{3}H-Tdr), a nucleoside precursor which is incorporated into newly synthesized DNA. The amount of ^{3}H-Tdr incorporated—and therefore the rate of DNA synthesis—is determined either by autoradiography and grain counts or by scintillation counting in a liquid scintillation spectrophotometer. The latter method is currently the generally accepted one in most clinical immunology laboratories.

Scintillation counting yields data in counts per minute (cpm) or corrected for quenching to disintegrations per minute (dpm), which are then used as a standard measure of lymphocyte responsiveness. The cpm in control cultures are either subtracted from or divided into stimulated cpm, which yields a ratio commonly referred to as the stimulation index.

Obviously there are a multitude of technical as well as conceptual variables that can affect the results of this sensitive assay system. These include the concentration of cells, the geometry of the culture vessel, contamination of cultures with nonlymphoid cells or microorganisms, the dose of mitogen, the incubation time of cultures, and the techniques of harvesting cells.

The degree of lymphocyte activation is also a function of the cellular regulatory influences present in the culture. Suppressor or helper T, B, and mononuclear cells are all capable of modifying the final degree of proliferation in the specifically stimulated cell population. Some mitogens, particularly Con A, are known to activate suppressor T cells, which may profoundly reduce the proliferative response in such cultures.

Of additional importance in lymphocyte activation are culture time and dose-response kinetics. Since clinically important defects in cellular immunity are rarely absolute, quantitative relationships in lymphocyte activation are of crucial importance. This is especially true when comparing the reduction of responsiveness of normal control subjects with a group of patients with altered lymphocyte function. With the use of microtiter culture systems and semiautomated harvesting devices, an attempt can be made to determine both dose- and time-response kinetics of either mitogen- or antigen-stimulated cultures (Figs 18–5 and 18–6).

Altered lymphocyte function can result in shifts in either time- or dose-response curves to the left or right. These shifts determine the optimal dose and optimal time of the lymphocyte response. Without such detailed analyses, it is usually impossible to accurately observe partial or subtle defects in lymphocyte responsiveness in various disease states. Cultures assayed at a single time with a single stimulant dose period are often grossly misleading.

Confusion may result from a nonstandardized format for presentation of data. Many laboratories present results of lymphocyte stimulation as a ratio of cpm in stimulated culture to those in control cultures—the so-called stimulation index. Others report "raw" cpm or dpm as illustrated in Figs 18–5 and 18–6. Neither method is entirely satisfactory. The stimulation index is a ratio, and marked changes can therefore result from changes in background or control cpm of the denominator. It is perhaps best to report data in both ways to permit better interpretations.

Table 18–6. "Nonspecific" mitogens that activate human lymphocytes

Mitogen	Abbreviation	Biologic Source	Relative Specificity
Phytohemagglutinin	PHA	*Phaseolus vulgaris* (kidney bean)	T cells
Concanavalin A	Con A	*Canavalia ensiformis* (jack bean)	T cells (different subset from PHA)
Antithymocyte globulin	ATG	Heterologous antisera	T cells
Staphylococcus protein A	SpA, SAC	*S aureus* (Cowan I strain)	B cells, T cell - independent
Pokeweed mitogen	PWM	*Phytolacca americana*	B cells, T cell -dependent
Streptolysin S	SLS	Group A streptococci	?(Probably T cells)

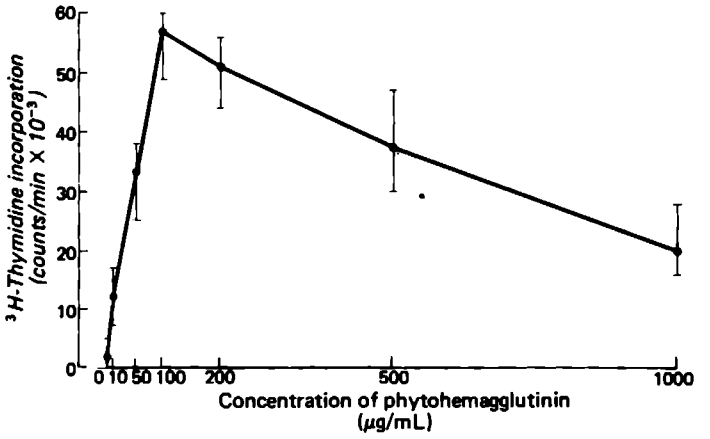

Figure 18–5. Dose-response curve for mitogen stimulation of 10^6 lymphocytes. Dose-response curve of a group of 10 normal adults whose peripheral blood lymphocytes were stimulated with varying concentrations of phytohemagglutinin for 72 hours. Lymphocytes were pulse-labeled with 2 μCi of tritiated (^{3}H) thymidine 6 hours prior to harvesting. Counts per minute of ^{3}H-thymidine incorporation were determined by liquid scintillation spectrometry and are plotted as the mean of 10 individual determinations ± the range. A maximal response occurred at approximately 100–200 μg/mL of phytohemagglutinin.

Antigen Stimulation

Whereas mitogens stimulate large numbers of lymphocytes, antigens stimulate far fewer cells which are specifically sensitized to the antigen in question. In most instances, only T cells respond to antigens in this test. A wide variety of antigens have been employed in lymphocyte activation, many of them also being used for delayed hypersensitivity skin testing (Table 18–7). In general, normal subjects show agreement between the results of skin tests and antigen-induced lymphocyte activation. However, in many conditions, the in vitro technique is apparently a more sensitive index of specific antigen-mediated cellular hypersensitivity.

Lymphocyte Culture Technique for Antigen Stimulation

Culture methods are virtually identical to those described for mitogen stimulation. Additional factors to be considered include the possible presence in serum supplements of antibody directed against stimulating antigens. Antigen-antibody complexes may block or occasionally nonspecifically stimulate lymphocytes.

As in the case of mitogen-induced activation, time- and dose-response kinetics are of crucial importance in generating reliable data. Representative examples of such curves are shown in Figs 18–7 and 18–8. In contrast to mitogen-induced lymphocyte activation,

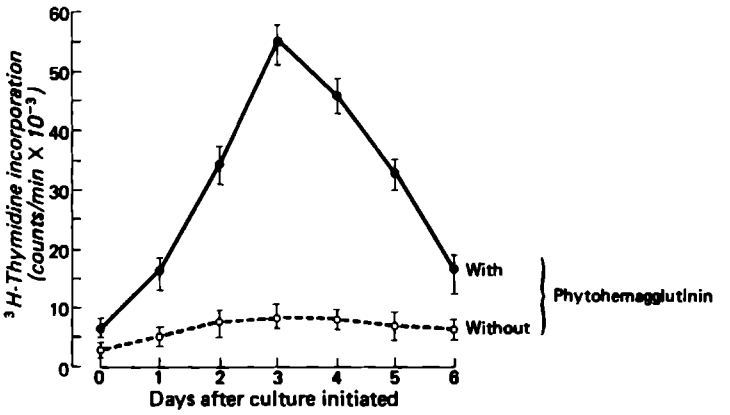

Figure 18–6. Time-response curve for mitogen stimulation of 10^6 lymphocytes. Time-response curve of peripheral blood lymphocytes from 10 normal adults stimulated in tissue culture for various lengths of time with an optimal concentration of phytohemagglutinin (100 μg/mL). Cultures were pulse-labeled with tritiated thymidine for 6 hours on the day of harvest. Maximal response occurred at 3 days after initiating the culture. Results are plotted as mean ± the range of counts per minute.

Table 18–7. Antigens commonly used to assess human cellular immunity in vitro.

PPD
Candida
Streptokinase/streptodornase
Coccidioidin
Tetanus toxid
Tumor antigens
Histoincompatible cells (mixed lymphocyte culture)
Vaccinia virus
Herpes simplex viruses

antigen stimulation results in lower total DNA synthesis. Furthermore, the time of maximal response does not occur until the culture has been allowed to continue for 5–7 days. Fig 18–8 clearly illustrates both the usefulness and the necessity of performing careful time- and dose-response kinetics in assessing human lymphocyte function.

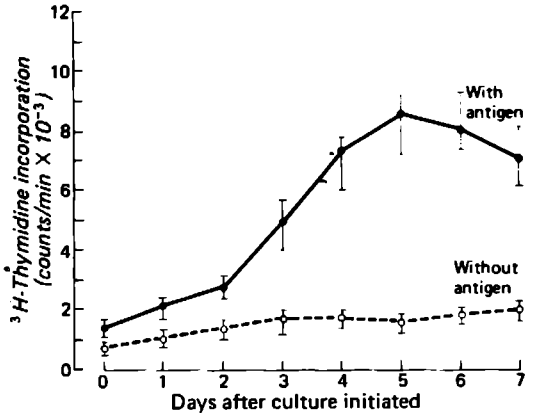

Figure 18–8. Time-response curve for antigen stimulation of 10^6 lymphocytes. Responses of peripheral blood lymphocytes from 15 normal adults with delayed hypersensitivity to the antigen. Cells were cultured as described in Fig 20–7. Antigen concentration for all cultures was 15 μg/mL. Maximal response occurred on days 5–7 of culture. Results plotted as mean ± the range for 15 individual determinations.

MIXED LYMPHOCYTE CULTURE & CELL-MEDIATED LYMPHOLYSIS

Mixed lymphocyte culture (MLC) is a special case of antigen stimulation in which T lymphocytes respond to foreign histocompatibility antigen on unrelated lymphocytes. This test is performed as either a "one-way" or "2-way" assay (Fig 18–9). In the one-way MLC, the stimulating cells are treated with either irradiation (~ 2000 R) or mitomycin to prevent DNA synthesis without killing the cell. The magnitude of the response is then entirely the result of DNA synthesis in the nonirradiated or nonmitomycin-treated cells. In the 2-way MLC, cells from both individuals are mutually stimulating and responding, and DNA synthesis represents the net response of both sets of cells and the individual contributions cannot be discerned. The culture conditions, time of exposure, ^{3}H-Tdr pulse labeling, and harvesting procedures are usually identical to those for antigen stimulation. Controls include co-culture of syngeneic irradiated and nonirradiated pairs and co-culture of allogeneic irradiated pairs. The first control provides baseline DNA synthesis and the sec-

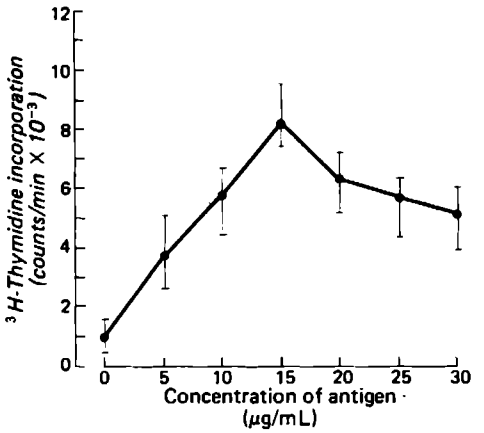

Figure 18–7. Dose-response curve for antigen stimulation of 10^6 lymphocytes. Dose-response curve of lymphocytes from 15 normal individuals with delayed hypersensitivity to the antigen. Cultures were harvested at 120 hours of culture after a 6-hour pulse with tritiated (^{3}H) thymidine. Counts per minute were determined by scintillation spectrometry. Results are plotted as mean ± the range from 15 skin test-positive subjects at various antigen concentrations. Maximum response is at 15 μg/mL of antigen.

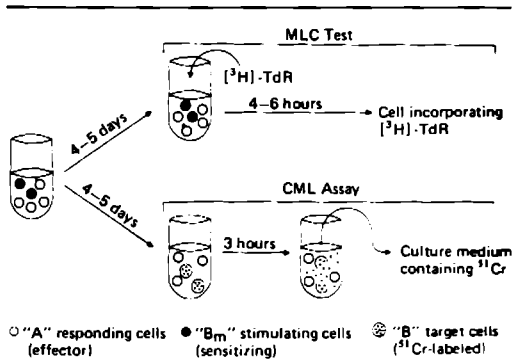

Figure 18–9. MLC and CML assays schematically represented. Cells (black and white balls) from separate individuals are cultured. In MLC, DNA synthesis in responding (noninactivated) cell is measured. In CML assay the ability of "A" cells to kill ^{51}Cr-labeled "B" cells is measured. See text for further explanation. (Reproduced with permission, from Bach FH, Van Rood JJ: The major histocompatibility complex: Genetics and biology. N Engl J Med 1976; 295:806, 872.)

ond assures adequate inactivation by irradiation (or mitomycin) of the stimulator cells.

In the use of MLC as a test for T cell function, difficulties in quantitation often arise owing to variations in stimulator cell antigens that determine the degree of genetic disparity between stimulator and responder cells. In order to overcome this difficulty and produce a more standardized test, frozen aliquots of *viable pooled* human allogeneic cells have been employed as stimulator cells.

There is currently great interest in the identity of stimulating and responding cells as well as the responsible cell-associated antigens in the MLC. It appears that the density of stimulating antigens (Ia-like) on human cells is higher on B lymphocytes and that these may be identical to HLA-D locus antigens (see Chapter 6). Responding cells are primarily T lymphocytes with obligate macrophage cooperation. B cells can also respond in MLC, since a marked increase in immunoglobulin synthesis can be detected. MLC may be used as a histocompatibility assay (Chapters 6 and 23) and as a test for immunocompetence of T cells, particularly in immunodeficiency disorders (Chapter 20).

Cell-mediated lympholysis (CML) is an extension of the MLC technique in which cytotoxic effector cells generated during MLC are detected (Fig 18–9). This test involves an initial one-way MLC culture followed by exposure of stimulated cells to ^{51}Cr-labeled target cells specifically lysed by sensitized killer lymphocytes. These target cells are HLA-identical to the stimulator cells in MLC. Cytotoxicity is measured as percentage of ^{51}Cr released in specific target cells compared to percentage of ^{51}Cr released from control (nonspecific) target cells. Several lines of evidence indicate that cells which proliferate in MLC and killer cells which participate in CML assay are not identical. Killer cells are generated that have specificity for HLA-A, HLA-B, or HLA-C antigens class I on target cells, whereas in MLC, HLA-D antigen class 2 differences determine the reaction. CML assays provide an additional measure of T cell function and can be used to estimate presensitization and histocompatibility in clinical transplantation. (See also Chapter 23.)

CLINICAL APPLICATION OF B & T CELL ASSAYS

Counting of B and T cells in peripheral blood and tissue specimens has widespread application in both the diagnosis and investigation of pathophysiologic mechanisms of many disease states. Current applications include the following:

(1) Diagnosis and classification of immunodeficiency diseases (Chapter 20).

(2) Determination of origin of malignant lymphocytes in lymphocytic leukemia and lymphoma (Chapter 22).

(3) Evaluation of immunocompetence and mechanisms of tissue damage in autoimmune disease, eg, systemic lupus erythematosus and rheumatoid arthritis (Chapter 21).

(4) Detection of changes in cellular immune competence in cancer that may be of prognostic value (Chapter 14).

(5) Monitoring of cellular changes following organ transplantation (Chapter 23).

NATURAL KILLER (NK) CELLS

Natural killer (NK) cells can be enumerated by specific monoclonal antibodies using methods identical to those for T and B cells. Several monoclonal antibodies are available that detect either Fc receptors (Leu 11) or specific differentiation antigens (Leu 7, HNK-1, NKH-1, NKH-2) present on these cells. Some NK cells also express antigens from the CD2 T cell family. Functional testing is done by ability of these nonimmune cells to kill special target cells such as erythroleukemia cell line K562. Cytotoxicity is usually performed using ^{51}Cr release assay (see below).

MONOCYTE-MACROPHAGE ASSAYS

The morphologic identification of normal peripheral blood monocytes in stained peripheral blood films ordinarily is quite simple. Monocytes are larger than granulocytes and most lymphocytes. They have round or kidney-shaped nuclei with fine, lightly stained granules. However, in suspension or even in tissue or blood specimens, additional markers may be required to differentiate monocytes from lymphocytes and primitive myeloid cells.

Perhaps the most reliable stain for monocytes is the so-called nonspecific esterase, or alpha-naphthol esterase, which is present in monocytes but absent in most myeloid and lymphocytic cells. Increasingly, monoclonal antibodies directed at specific differentiation antigens are becoming available. These include Leu M3, OKT M1 (also present in some T cells), and others. It has generally been difficult to produce highly specific monocyte antibodies or define subsets of monocytes with such antibodies.

Functional attributes of monocytes are discussed in detail in Chapter 9. In the clinical laboratory, phagocytosis of particles or antibody-coated heat-killed microorganisms is a convenient test for functional identification of monocytes.

NEUTROPHIL FUNCTION

Polymorphonuclear neutrophils (PMNs) are bone marrow-derived white blood cells with a finite life span which play a central role in defense of the host against infection. For many types of infections, the neutrophil plays the primary role as an effector or

killer cell. However, in the bloodstream and extravascular spaces, neutrophils exert their antimicrobial effects through a complex interaction with antibody, complement, and chemotactic factors. Thus, in assessing neutrophil function, one cannot view the cell as an independent entity; its essential dependence on other immune processes, both cellular and humoral, must be taken into account.

Defects in neutrophil function can be classified as quantitative or qualitative. In quantitative disorders, the total number of normally functioning neutrophils is reduced below a critical level, allowing infection to ensue. Drug-induced and idiopathic neutropenia (see Chapter 22) with absolute circulating granulocyte counts of less than 1000/μL are examples of this sort of defect. In these situations, granulocytes are functionally normal but are present in insufficient numbers to maintain an adequate defense against infection. In qualitative neutrophilic disorders, the total number of circulating PMNs is either normal or sometimes actually elevated, but the cells fail to exert their normal microbicidal functions. Chronic granulomatous disease is an example of this type of disorder (see Chapter 20). The cause of chronic granulomatous disease is unknown; what is known is that the normal or increased numbers of circulating neutrophils in such patients are unable to kill certain types of intracellular organisms.

Phagocytosis by PMNs can be divided into 5 distinct and temporally sequential stages: (1) motility, (2) recognition, (3) ingestion, (4) degranulation, and (5) intracellular killing (Fig 18–10). The microbicidal activity of the neutrophil is the sum of the activity of these 5 phases. The clinical syndromes resulting from defects in many of the various stages in phagocytosis are discussed in Chapter 20. The laboratory tests used in clinical practice to evaluate phagocytic function in humans with various diseases will be discussed in terms of the 5 major steps in the process. It should be emphasized that for many neutrophil functions no standard assay exists. The following sections will include examples of useful clinical tests of neutrophil function.

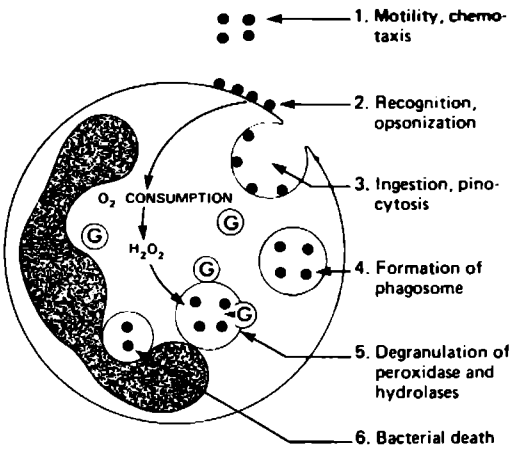

Figure 18–10. Steps in progress of phagocytosis. Schematic representation of phagocytosis by a granulocyte. 1: Bacteria attract phagocytic cells by chemotactic stimulus. 2: Presence of opsonins (immunoglobulin and complement) facilitates recognition and surface attachment. 3: Invagination of cell membrane with enclosed opsonized bacteria. 4: Intracellular organelle, the phagosome, forms. 5: Granules fuse with phagosomes and release enzymes into the phagolysosome. 6: Bacterial death and digestion result. (Modified from Baehner: Chronic granulomatous disease. Page 175 in: *The Phagocytic Cell in Host Resistance*. Bellanti JA, Dayton DH [editors]. Raven Press, 1975.)

TESTS FOR MOTILITY

Neutrophils are constantly in motion. This movement can be either random or directed. Random or passive motion is the result of **brownian movement.** In **chemotactic movement** the cells are actively attracted to some chemotactic stimulus. Chemotaxins are produced by complement activation (C3a, C5a, C567; see Chapter 10), by microorganisms themselves (endotoxins), and by other leukocytes (lymphocyte chemotactic factor). Relatively simple assays have been designed to assess leukocyte movement in vitro. Actually, an in vivo technique, the Rebuck skin window, preceded the development of in vitro assays and was one of the earliest methods developed for assessing leukocyte function.

Test for Random Motility

Random motility is tested for by the **capillary tube method.** Purified neutrophils in 0.1% human albumin solution at a concentration of 5×10^6/mL are placed in a siliconized microhematocrit tube. The tube is enclosed in a chamber specially constructed from microscope slides and embedded in adhesive clay. After being filled with immersion oil, the entire chamber is placed on the stage of a microscope. Motility is assessed by observing the leading edge of the leukocyte column in the microscope at hourly intervals. Measurements are expressed in millimeters of movement from the starting boundary of the packed leukocyte layer.

Test for Chemotaxis

Quantitation of directional locomotion of neutrophils toward various chemotactic stimuli is accomplished by use of a Boyden chamber. Cells to be tested are placed in the upper chamber and are separated from the lower chamber containing a chemotactic substance by a filter membrane of small pore size. Neutrophils can enter the filter membrane but are trapped in transit through the membrane. After a suitable incubation period, the filter is removed and stained and the underside is microscopically examined for the presence of neutrophils.

Although this method is theoretically simple, there are numerous technical difficulties. These include

nonavailability of filters of standard pore size, observer bias in quantitation of migrating neutrophils in the microscope, loss of cells which fall off or completely traverse the filter, and failure of many workers to standardize cell numbers and serum supplements.

An additional method for measuring chemotaxis and random motility has been recently developed. This technique involves the radial migration of leukocytes from small wells cut into an agarose medium in a Petri dish. In many respects, the method is similar to single radial diffusion (see Chapter 17). Generally, 3 wells are cut into agarose. The cell population in question is placed in the center well. A chemoattractant is placed in an outer well, and a control nonattractant is placed in the remaining well. After several hours of migration, the distance from the center of the well originally containing cells to the leading edges of the migrating cells is measured. In this way, the directed motility and the random motion can be quantitated. This method has achieved widespread application and in many laboratories has supplanted the somewhat more cumbersome Boyden chamber technique.

TESTS FOR RECOGNITION

As the neutrophil in an immune host approaches its target, by either random or directed motility, it recognizes microorganisms by the presence of antibody and complement fixed to the surface of the microorganisms. Enhancement of phagocytosis (opsonization) occurs under these circumstances.

Tests to detect the presence of complement and antibody Fc receptors on neutrophils are research tools and have no direct clinical applications. The need for either antibody or complement (opsonins) coating of microorganisms for phagocytosis can be determined by employing sera devoid of either or both of these factors followed by an assay for ingestion and subsequent intracellular killing. Furthermore, IgG and complement receptors on neutrophils as well as mononuclear phagocytes can be readily detected by rosette formation with IgG-coated or complement-coated erythrocytes. Such assays have not been widely applied clinically in the study of failure of host defenses against infection but have considerable promise for elucidating defects in the recognition phase of phagocytosis.

TESTS FOR INGESTION

Ingestion of microorganisms by neutrophils is an active process that requires energy production by the phagocytic cell. Internalization of antibody-coated and complement-coated microorganisms occurs rapidly following their surface contact with neutrophils. Since subsequent intracellular events—ie, degranulation and killing—depend on the success of ingestion, tests for ingestion provide a rapid and relatively simple means of assessing the overall phagocytic process. Unfortunately, the term phagocytosis has often been used to denote *only* the ingestion phase of the process. Thus, terms such as phagocytic index which refer to the average number of particles ingested really should be considered measurements of ingestion rather than of phagocytosis.

All tests to measure the ability of neutrophils to ingest either native or opsonized particles employ 2 general approaches. Either a direct estimate is made of the cellular uptake of particles by assaying the cells themselves, or the removal of particles from the fluid or medium is taken as an indirect estimate of cellular uptake.

Methods for quantitation of the ingestion of particles by cellular assays include (1) direct counting by light microscopy; (2) estimation of cell-bound radioactivity after ingestion of a radiolabeled particle; and (3) measurement of an easily stained lipid, eg, oil red O, after extraction from cells.

One disadvantage of many of these assays is that particles adherent to the neutrophil membrane are included as ingested particles. Other elements that influence results in performing ingestion assays include the presence of humoral factors (opsonins) which enhance uptake, the presence of serum containing acute-phase reactants which depress uptake, the need for constant agitation or tumbling of cells and particles to maximize contact and subsequent uptake, the type or size of the test particle used, and, finally, the ratio of particles to ingesting cells. No well-standardized assay is currently available for estimating particle ingestion.

TESTS FOR DEGRANULATION

Following ingestion of particles or microorganisms, the ingested element is bound by invaginated cell surface membrane in an organelle termed the **phagosome.** Shortly thereafter, lysosomes fuse with the phagosome to form a structure called the **phagolysosome.** Degranulation is the process of fusion of lysosomes and phagosomes with the subsequent discharge of intralysosomal contents into the phagolysosome.

Degranulation is an active process and requires energy expenditure by the cell. Thus, impairment of normal metabolic pathways of the neutrophil—especially oxygen consumption and the metabolism of glucose through the hexose monophosphate shunt—interferes with degranulation and subsequent intracellular killing.

A test for degranulation called frustrated phagocytosis has been developed and applied to the study of some neutrophil dysfunction syndromes. The frustrated phagocytosis system (Fig 18–11) allows for examination of degranulation independently of ingestion. Heat-aggregated γ-globulin or immune complexes are fixed to the plastic surface of a Petri dish so that they cannot be ingested. Neutrophils are placed in suspension in Petri dishes with and without

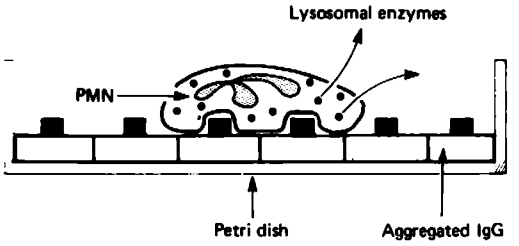

Figure 18–11. Assay of granulocyte degranulation by the "frustrated phagocytosis" method. Neutrophil is attached to aggregated IgG fixed to bottom of Petri dish. Lysosomal enzymes are discharged into supernate as the cell attempts to phagocytize the IgG but is "frustrated." (Courtesy of S Gold.)

attached aggregated γ-globulin. The cell membranes of the neutrophils are stimulated by contact between γ-globulin and appropriate cell membrane receptors. This process results in fusion of intraleukocyte granules (lysosomes) with the cell membrane. As a result, intralysosomal contents are discharged into the suspending medium. The rate of release of lysosomal enzymes, particularly β-glucuronidase and acid phosphatase, is taken as an estimate of the rate of degranulation. Nonspecific cell death or cytolysis can be estimated by measuring the discharge of lactate dehydrogenase (a nongranule enzyme) into the medium. This assay system has been used to demonstrate retardation in the degranulation rate by neutrophils from patients with chronic granulomatous disease.

TESTS FOR INTRACELLULAR KILLING

The primary function of the neutrophil in host resistance is intracellular killing of microorganisms. This final stage of phagocytosis is dependent on the successful completion of the preceding steps: motility, recognition, ingestion, and degranulation. A variety of intraleukocytic systems make up the antimicrobial armamentarium of the neutrophil (Table 18–8). Obviously, a defect in intracellular killing could be the result of any one or a combination of these functions. However, in clinical practice only 2 assays have received widespread use, ie, the nitroblue tetrazolium dye reduction test and the intraleukocytic killing test. It is hoped that specific metabolic and antimicrobial assays for other intraleukocytic events will also become available in future.

Nitroblue Tetrazolium Dye Reduction Test

Nitroblue tetrazolium (NBT) is a clear, yellow, water-soluble compound that forms formazan, a deep blue dye, on reduction. Neutrophils can reduce the dye following ingestion of latex or other particles subsequent to the metabolic burst generated through the hexose monophosphate shunt. The reduced dye can be easily measured photometrically after extraction from neutrophils with the organic solvent pyridine. The reduction of NBT to a blue color thus forms the basis of the quantitative NBT test. The precise mechanism of NBT reduction is not known, but the phenomenon is closely allied to metabolic events in the respiratory burst following ingestion, including increased hexose monophosphate shunt activity, increased oxygen consumption, and increased hydrogen peroxide and superoxide radical formation. Since the generation of reducing activity in intact neutrophils parallels the metabolic activities following ingestion, NBT reduction is a useful means of assaying overall metabolic integrity of phagocytizing neutrophils. Failure of NBT dye reduction is a consistent and diagnostically important laboratory abnormality in chronic granulomatous disease. Neutrophils from these patients fail to kill certain intracellular microbes and fail to generate H_2O_2 or the superoxide radical.

Quantitative NBT Test

Isolated neutrophils are incubated in a balanced salt solution with latex particles and NBT. After 15 minutes of incubation at 37 °C, the reduced dye (blue formazan) is extracted with pyridine and measured spectrophotometrically at 515 nm. The change in absorbance between cultures of cells that actively phagocytize latex particles and those that do not is taken as an index of neutrophil function. The test is strikingly abnormal in chronic granulomatous disease (see Chapter 20). Various modifications of the quantitative NBT test have been developed as screening tests for chronic granulomatous disease. Prominent among these are so-called slide tests in which neutrophils, latex, and NBT are placed in a drop on a glass slide and the reduction to blue formazan assayed under the microscope. It can be performed on a single drop of blood, but abnormal results should be confirmed with the more precise quantitative method described above.

NBT Tests in Diagnosis of Bacterial Infection

Since NBT dye reduction is enhanced by ingestion

Table 18–8. Antimicrobial systems of neutrophils.*

Acid pH of phagolysosome
Lysozyme
Lactoferrin
Cationic proteins
Myeloperoxidase-halogenation system
Hydrogen peroxide
Superoxide radical
Hydroxyl radical
Singlet oxygen

*For a further description of these systems, see Klebanoff SJ: Antimicrobial mechanisms in neutrophilic polymorphonuclear leukocytes. *Semin Hematol* 1975;**12**:117 and Cheson BD, Curnette JT, Babior BM: The oxidative killing mechanisms of the neutrophil. *Prog Clin Immunol* 1977;**3**:1.

of latex particles, it has been reasoned that dye reduction should also be enhanced by phagocytosis of bacteria in infected humans. However, initial enthusiasm for the use of simple spontaneous NBT dye reduction assays, usually performed by the slide method, has been tempered by reports of false-positive or false-negative results in numerous diseases.

The NBT test performed by quantitative assay should be reserved for the diagnosis of chronic granulomatous disease, for the study of factors involved in phagocytosis, and for the detection of patients with intraleukocytic killing defects.

Chemiluminescence

Neutrophils emit small amounts of electromagnetic radiation following ingestion of microorganisms. This energy can be detected as light by sensitive photomultiplier tubes, such as those in liquid scintillation counters. During the respiratory burst, H_2O_2, superoxide radicals, and singlet oxygen are generated. Singlet oxygen, a highly unstable and reactive species, combines with bacteria or other intralysosomal elements to form electronically unstable carboxyl groups. As these groups relax to ground state, light energy is emitted. This entire process has been termed **chemiluminescence** and forms the basis of an important assay of neutrophil function. Similar to NBT, it requires all steps prior to actual bacterial killing to be intact. Recent studies show a precise correlation between light emissions and microbicidal activity. The oxidative steps in the biochemical pathways present in the neutrophil generate the chemiluminescence, which is easily detected in a liquid scintillation spectrometer with the coincidence circuit excluded.

In the test, neutrophils are incubated in clear, colorless balanced salt solution in the presence of an ingestible particle, eg, latex or zymosan, in a scintillation vial. Luminol, an intermediate fluorescent compound, can be added to intensify the light emissions. The emission of photons of light is measured as cpm in a scintillation counter over the next 10 minutes at 2-minute intervals. Studies with this technique have revealed markedly reduced chemiluminescence in chronic granulomatous disease (patients and carriers) and in myeloperoxidase-deficient patients. This method appears to be somewhat more sensitive than the quantitative NBT test and can probably be performed on very small numbers of cells. Newer methods employ a whole-blood method that greatly simplifies the procedure by obviating the granulocyte separation steps. Many laboratories are substituting it for NBT reduction as a screening test for neutrophil dysfunction and in detection of carriers of chronic granulomatous disease.

Neutrophil Microbicidal Assay

Many strains of bacteria and fungi are effectively engulfed and killed by human neutrophils in vitro. Assuming that all of the stages of the phagocytic process that precede killing within the phagolysosome are intact, microbicidal assays are extremely useful tests for neutrophil function. As an example, the bactericidal capacity of neutrophils for the common test strain 502A of *S aureus* will be described in some detail.

Bacteria are cultured overnight in nutrient broth to make certain that they will be in a logarithmic growth phase. They are then diluted to give about 5 bacteria per neutrophil in the final test. Neutrophils are separated from whole heparinized blood by dextran sedimentation and lysis of red blood cells with 0.84% NH_4Cl. Opsonin is provided as a 1:1 mixture of pooled frozen serum (−70 °C) and serum from freshly clotted blood. Bacteria, neutrophils, and opsonin are incubated in tightly capped test tubes and tumbled end over end at 37 °C. An aliquot of the entire mixture is sampled at 0 time. After 30 minutes of incubation, antibiotics are added to kill extracellular bacteria. Aliquots of neutrophils with ingested organisms are sampled at 30, 60, and 120 minutes. Intracellular microorganisms are liberated by lysis of neutrophils by sterile water and the number of *viable* intracellular bacteria is estimated by serial dilutions and plating of lysed leukocytes. Results plotted as in Fig 18–12 show that normal neutrophils result in an almost 2-log reduction in viable intracellular *S aureus* 1 hour after incubation. Killing is virtually absent in cells from patients with chronic granulomatous disease and intermediate in heterozygous carriers of these inherited diseases.

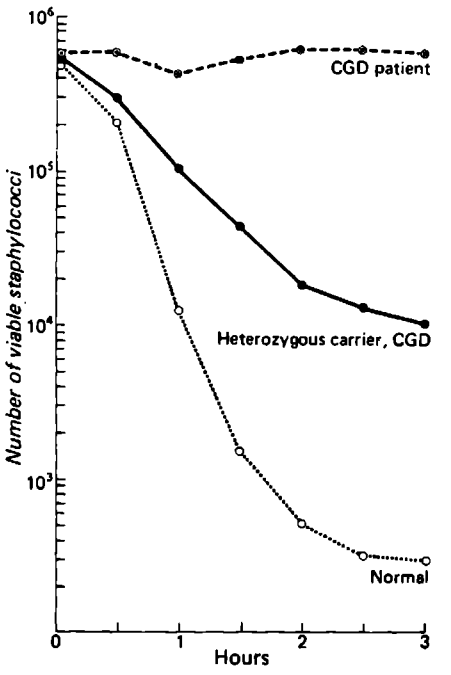

Figure 18–12. Bactericidal assay of granulocytes. Curves represent number of viable intracellular organisms that survive after being ingested by granulocytes. Note marked decline in bacterial survival in normal cells compared to reduced to absent killing by cells from patients and relatives with CGD (chronic granulomatous disease).

By varying the test organism or the source of opsonin, this assay can be effectively used to measure a wide range of microbicidal activities and serum-related defects. Obviously, falsely "normal" killing will be the interpretation of the results if cells fail to ingest organisms normally. Thus, an independent assay for microbial ingestion must be performed prior to the neutrophil microbicidal test.

Some diseases with defective microbicidal activity demonstrable with this assay are listed in Table 18–9. For further details, see Chapter 20.

Table 18–9. Disorders of neutrophil function.

Chronic granulomatous disease (X-linked or autosomal recessive)
Job's syndrome
Chédiak-Higashi syndrome
Myeloperoxidase deficiency
Glucose-6-phosphate dehydrogenase deficiency
Acute leukemia
Down's syndrome
Premature infants
Transient neutrophil dysfunction
Acute infections
Ataxia-telangiectasia
Cryoglobulinemia

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19 Blood Banking & Immunohematology

Juhani Leikola, MD

Although blood transfusion has become routine in clinical practice, it still imposes definite immunologic and infectious hazards. Most of the risks can be avoided by careful testing of both blood donor and recipient before the transfusion. The emergence of acquired immunodeficiency syndrome (AIDS) as a risk factor for transfusion of blood, blood components, and coagulation factor concentrates has emphasized that infectious diseases are still the main cause of severe transfusion complications. Careful attention to the immunologic and infectious problems of blood transfusion reduces the inherent risks, but the hazards of transfusion cannot be totally eliminated. It is therefore necessary to weigh the therapeutic benefits against the potential hazards before proceeding with the transfusion.

BLOOD GROUPS

The surface of red blood cells contains large numbers of antigenic determinants that are direct or indirect products of genes. These antigenic determinants are classified into blood groups. Within each blood group system, antigens appear to be inherited as products of a single gene or group of closely linked genes. The number of recognized blood groups increases every year (Table 19–1).

The chemical structure of most of these antigens remains unknown, although the nature of some erythrocyte membrane proteins has been established. The research has focused mainly on substances called sialoglycoproteins or glycophorins. Of these, glycoprotein-α (glycophorin A) carries the blood group M and N antigens and glycoprotein-δ (glycophorin B) the Ss and U antigens. Other glycoproteins have A and B activity. The complete amino acid sequence and carbohydrate composition of glycoprotein-α is now known, and studies to establish the detailed structure of glycoprotein-δ are well under way. Some antigens of cold-reacting autoantibodies reside also in the various membrane glycoproteins.

The clinical significance of a blood group system depends on 2 factors: the frequency of antibodies in the population and their relative potency. Even if many blood groups are not important in clinical transfusion practice, they are objective, simply inherited genetic markers useful in anthropologic studies and in disputed paternity cases.

Table 19–1. Established blood group systems.

System	Symbol	Number of Antigens for Which Specific Antibody is Known	Chromosomal Location*
ABO (H)		5	9 (19)
MNSs		30	4
P		2	?
Rhesus	(Rh)	44	1
Lutheran	(Lu)	16	19
Kell		21	?
Lewis	(Le)	2	19
Duffy	(Fy)	5	1
Kidd	(Jk)	3	2
Diego	(Di)	2	?
Cartwright	(Yt)	2	?
Auberger	(Au)	1	?
Xg		1	X
Scianna	(Sc)	3	1
Dombrock	(Do)	2	?
Colton	(Co)	3	2
Landsteiner-Weiner	(LW)	3	19
Low-incidence antigens†		39	NA
High-incidence antigens†		28	NA
Total		212	

*All antigens within a system have not been shown to be controlled by the same locus or even by closely linked loci.
†Not assigned to any of the systems.

ABO & LEWIS GROUPS

ABO Antigens

In 1901, Landsteiner published his observation of a consistent pattern in agglutination of human blood cells by other human sera. Cells from group A persons were agglutinated by serum from group B individuals and vice versa (Table 19–2). Cells from others (group O) were not agglutinated by any sera. These people had antibodies to both A and B. Soon after Landsteiner's original discovery, the fourth group, AB, was found with no antibodies to other human cells.

A and B are carbohydrate antigens. The antigenic specificity resides in the terminal sugars of an oligosaccharide. On cell membrane, the majority of A and B antigens are on glycoproteins, but some of the carbohydrate is also bound on membrane lipids. A and B antigens may also appear in body fluids as soluble glycoproteins.

Table 19–2. Blood group antigens and antibodies in ABO system.

Blood Group	Antigen on Red Cells	Antibodies in Plasma	Frequency*		
			Caucasian	Black	Oriental
A_1	$A + A_1$	anti-B			
A_2	A + H	anti-B ($anti\text{-}A_1$)	41	28	38
B	B (+H)	anti-A	11	17	22
O	H	anti-A anti-B	45	51	30
A_1B	$A + A_1 + B$	...	3	4	10
A_2B	A + B (+ H)	($anti\text{-}A_1$)			

*Frequencies vary in different populations, and the given figures have to be considered as examples.

The structure of A and B is schematically shown in Fig 19–1. The last 3 sugars in this stem chain—called H substance—are N-acetylglucosamine, galactose, and fucose. N-Acetylgalactosamine in blood group A and galactose in blood group B are bound to the last galactose of the stem chain.

The ABO blood groups are determined by allelic genes A, B, and O. These genes produce transferase enzymes that conjugate the terminal sugars to the stem carbohydrate. These transferases are specific for their substrates: A transferase for N-acetylgalactosamine, and B transferase for galactose. O-gene produces no transferase that would modify the blood group substance. Group O persons have only the stem substance, H antigen. In the absence of direct transferase determinations or family studies, it is not possible to determine whether a person who belongs to group A is homozygous, with two A genes, or is heterozygous, with one A and one O gene (Fig 19–2).

The stem chain is influenced by a transferase that conjugates fucose to galactose. This transferase is a product of H gene that is inherited independently of the ABO genes. There are rare persons who lack H genes (genotype hh), thus being unable to synthesize a complete stem chain. This phenotype is called O_h or Bombay type. Because of deficient stem chain, A and B substances cannot be formed despite functional A and B transferases.

Blood group A can be divided into subgroups A_1 and A_2 on the basis of the number of A antigenic sites on the cell. About four-fifths of A and AB persons belong to subgroup A_1. The high antigen density on A_1 cells results in formation of a new but relatively weak antigenic specificity, perhaps as a result of the interaction of 2 closely adjacent A-oligosaccharides. A_2 and especially A_2B persons may have antibodies to A_1.

There are rare subtypes of A that have much less A substance than even A_2 cells. A_2 cells react characteristically with anti-A with very small agglutinates surrounded by a large number of nonagglutinated cells. These individuals probably have a heterogeneous red cell population, some cells with nearly normal amounts of A, and many cells with no detectable antigen. A_x cells are not agglutinated by anti-A derived from group B persons; however, serum from group O individuals characteristically produces a weak reaction. To be able to detect A_x and some other still rarer subgroups of A, blood grouping laboratories often use O serum (called anti-A,B) in addition to anti-A and

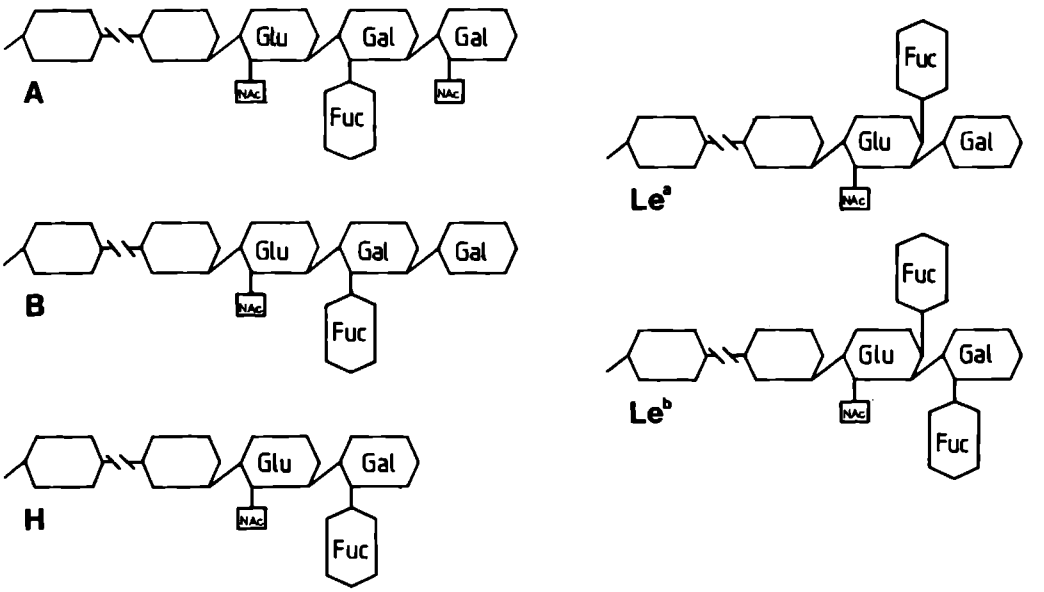

Figure 19–1. Diagrams of proposed chemical structure of blood group antigens A, B, H, Le^a, and Le^b. The sugars are linked by carbohydrate chains to either a peptide chain or a lipid. Glu = glucose; Gal = galactose; Fuc = fucose. NAc = N-acetyl group.

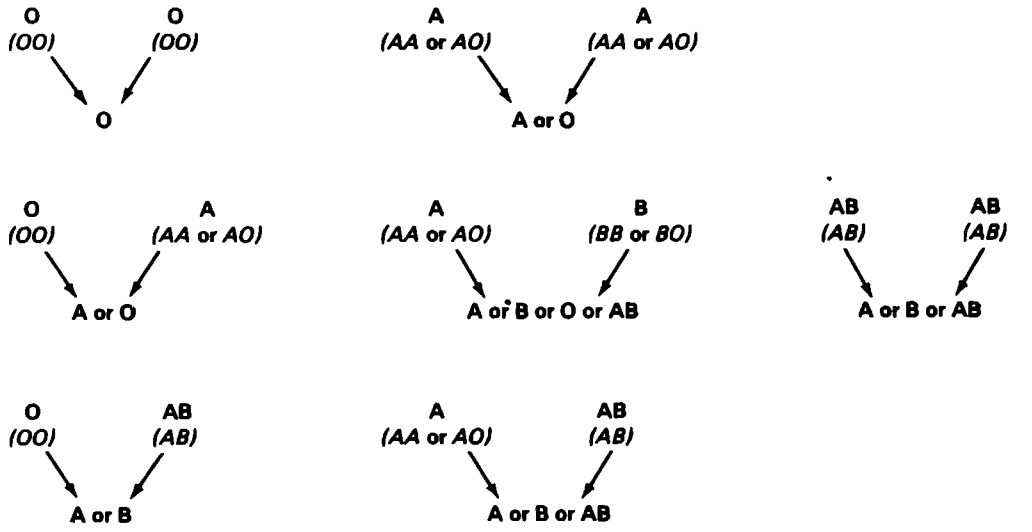

Figure 19–2. Simplified examples of inheritance of ABO groups. Blood group phenotype is printed in nonitalics and genotype in italics.

anti-B. Blood group B cannot be divided into subgroups, but there are some very rare forms of weak B.

Since the antibodies to A_1 are weak, they are not always practical for differentiating between A_1 and A_2. The distinction is usually made by using substances called lectins. These plant seed extracts selectively react with some carbohydrate blood group antigens. The lectin from *Dolichos biflorus*, in appropriate dilution, clumps only A_1 cells. It is often used simultaneously with lectin from *Ulex europaeus*, which reacts with H substance, since A_2 cells have plenty of H substance compared to A_1 cells.

Anti-A & Anti-B

The biochemical structures of the blood group oligosaccharides occur commonly in nature. Substances derived from intestinal bacteria and some ingested vegetables can trigger an immune response in humans. This stimulation is thought to produce anti-B and anti-A in A, B, and O people. Infants start producing anti-A or anti-B (sometimes called isoagglutinins) after a few months of life. In patients with humoral immune deficiency, like hypogammaglobulinemia, the amount of anti-A or anti-B may be reduced to undetectable levels. Anti-H (in addition to anti-A and anti-B) is always present in the serum of the rare O_h individuals, and it is a relatively common specificity in cold-reacting autoantibodies.

Anti-A and anti-B are usually IgM, but a sufficient immunologic stimulus can result in formation of IgG anti-A and anti-B. IgG antibodies can cross the placenta and may cause destruction of fetal red cells. Attempts have been made at antenatal screening of maternal IgG anti-A and anti-B, but the correlation with clinical hemolytic disease of the newborn is not very good (see Chapter 22).

Anti-A and anti-B may be hemolyzing, and they are stronger in group O persons than in A or B individuals. Group O plasma should not be transfused into A or B patients in large amounts. Therefore, group O red cell concentrate containing only small volumes of plasma should be used instead of whole blood if there is not time to type the patient or if blood of the patient's own group is not available.

Secretors

In about 80% of people, soluble blood group antigens appear in saliva, milk, and other body fluids. The secretion of ABH substances is regulated by allelic genes Se and se that are independently inherited from the ABO system. Secretor status is a mendelian dominant character.

Blood group substances in saliva are detected by hemagglutination inhibition after boiling and centrifugation to remove enzymes and other proteins. ABH antigens are very stable, and the demonstration of blood group substances in cigarettes, sweat spots, etc, is used as evidence in criminal prosecutions. Except for Lewis- and Sd^a-antigens, other blood groups are not present in saliva.

Lewis Groups

The genes regulating the Lewis blood group system are not linked to an ABO locus, but they probably are linked to H and Se loci, and the Le antigens are structurally closely related to ABH substances. The stem carbohydrate is essentially the same in ABH and Le. In the presence of Le gene, a transferase is formed that conjugates a fucose to N-acetylglucosamine in the stem chain (Fig 19–3). Whether the resulting antigen is Le^a or Le^b depends on the secretor status of the individual. If the subject secretes blood group substances,

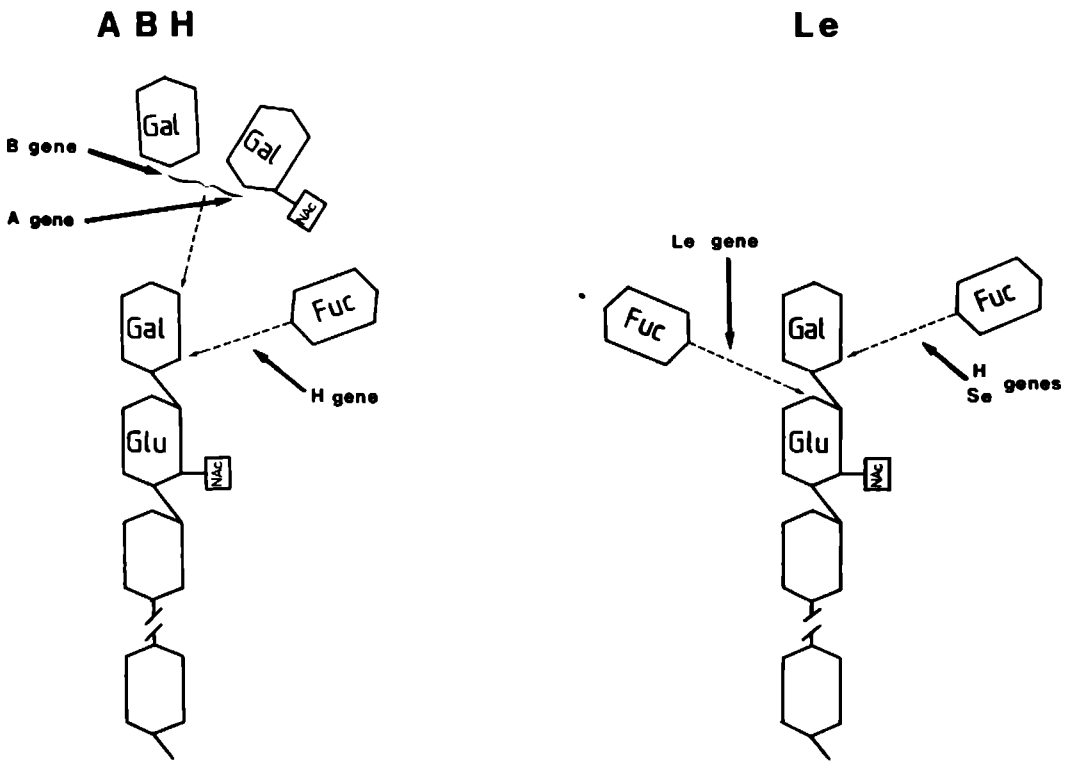

Figure 19–3. Proposed modification of blood group precursor substance by genes of ABO, secretory (Se), and Lewis (Le) systems. Dashed arrows indicate the action of specific transferases, coded by polymorphic genes. For attachment of fucose to galactose in H and Lewis substances, function of both H and Se genes is probably needed. Lewis substance is passively adsorbed by red cells. Gal = galactose; GalNAc = N-acetylgalactosamine; GluNAc = N-acetylglucosamine, Fuc = fucose.

the antigen is Le^b; if a nonsecretor, the antigen is Le^a. About 20% of Caucasians are group Le(a+), 75% are Le(b+), and the rest are Le(a−b−).

Soluble Lewis substances occur in saliva and plasma. In fact, the antigens on red cells have been passively adsorbed from the surrounding plasma. Le(a−b−) erythrocytes can be rendered Lewis-positive by incubating them in Le(a+) or Le(b+) plasma. The expression of Le^b on red cells is influenced by the ABO group so that O and B persons have more Le^b than A_1 individuals. This variation of antigenic strength makes saliva more suitable for Le^b typing than red blood cells.

Lewis antibodies can occur naturally without antigenic stimuli from other persons' red cells. The antibodies are usually weak and almost exclusively IgM class. A strong anti-Le^a may cause transfusion problems, but anti-Le^b is generally harmless. They do not cause hemolytic disease of the newborn. Only Le(a−b−) persons are able to form anti-Lewis antibodies.

Rh GROUPS

In 1939, Levine and Stetson found that the serum of a mother who had delivered a dead baby agglutinated erythrocytes of about 85% of human adults. The next year, Landsteiner and Wiener published their observations on immunization of guinea pigs and rabbits with rhesus monkey blood. They obtained, after appropriate absorptions, an antibody that would agglutinate a similar proportion of human erythrocytes. The group was given the name Rh, although the rhesus monkey antigen was much later shown to differ from the human red cell antigen and was renamed LW to honor Landsteiner and Wiener.

Rh Antigens

With the discovery of new antibodies and antigens, the immunology and genetics of Rh groups have become very complicated—over 40 different specificities have been found. The precise chemical nature of these antigens remains unknown.

There are 2 genetic theories to explain the multispecificity of the Rh antigen complex. According to the theory of Fisher and Race, the Rh antigens are determined by 3 pairs of closely linked genes; the theory

proposed by Wiener suggests a high number of allelic genes at one locus determining the whole complex. Unfortunately, the proponents of these 2 competing theories used different nomenclatures. It has also been suggested that all Rh antigenic specificities should be expressed as numerals. Over 40 years after the discovery of the Rh blood group system, considerable confusion still exists in the field because of conflicting systems of terminology.

In practice, many laboratories use a compromise between the nomenclatures. The original Rh antigen is called D, and it is accompanied by the gene products of Cc and Ee loci. The counterpart of D is probably not antigenic, or the gene d is amorphic, since antibodies to d have never been found. In any population, these antigens do not occur at random but have preferential combinations. By using different antisera to determine the Rh phenotype, the probable genotype can be inferred. The genotype is described by 2 sets of letters, eg, CDe/cDE, or more commonly by using only 2 letters, eg, R^1R^2 (Table 19–3). If only anti-D serum is used in testing, it is not possible to distinguish between homozygous and heterozygous Rh-positive persons. This distinction is clinically important when the father's blood is typed in a case of Rh hemolytic disease of the newborn.

Of the various Rh antigens, D is the strongest immunogen and therefore the most important. It provokes antibody response about 50 times more frequently than the antigens c and E. If one unit of D-positive blood is transfused to an Rh-negative recipient, anti-D will be produced in 60–80% of cases. Therefore, only the D antigen is determined in routine blood banking, and its presence determines the designation Rh-positive or Rh-negative.

Antigen D can appear on the red cell membrane in weak forms that complicate Rh typing. These variants are called D^u, and they differ from "normal" D by having a smaller number of antigenic sites. The detection of D^u requires a strong antiserum and Coombs technique when testing is done manually (see Chapter 17). If D^u is detected, the blood is considered Rh-positive.

In rare cases, the red blood cells do not react with any Rh antisera. The whole Rh substance is lacking, and the group is called Rh_{null}. The antibodies in autoimmune hemolytic anemia (see Chapter 22) often react with all other cells except for Rh_{null}. Therefore, the antibody is said to have Rh specificity.

Rh Antibodies

Since Rh antigens occur only on red cells, there are no "natural" Rh antibodies. An immune response is elicited only after exposure to incompatible blood, either in pregnancy or by transfusion, although some autoantibodies have Rh-like specificities.

Anti-D is the commonest and strongest Rh antibody. As little as 0.01 mL of Rh-positive blood may be enough to arouse antibody formation. Since Rh antigens appear early in ontogeny, immunization after abortion of an Rh-positive fetus is possible, although the risk is 10–20 times less than after full-term pregnancy.

Rh antibodies may be IgM initially after a strong immunization, but usually they are IgG. There are only 10,000–30,000 Rh antigen sites on each red cell, compared to about 1 million A_1 sites. Because of this low density of antigenic sites, relatively few antibody molecules can be bound onto the cell surface. If the antibodies are IgG class, the cells are not agglutinated in normal physiologic saline, since there cannot be enough Rh antibody molecules to counteract the repulsive forces that hold the cells as a suspension. In contrast, the larger IgM antibodies can bridge the cells together. IgG anti-Rh antibodies are called **incomplete** and IgM anti-Rh **complete** or saline-agglutinating antibodies.

Agglutination by IgG antibodies can be enhanced in 3 ways: (1) by using antiserum to IgG (Coombs technique; see Chapter 17); (2) by adding 20–30% bovine serum albumin (BSA) to the reaction mixture; and (3) by treating the cells with a proteolytic enzyme (eg, papain or bromelain). Addition of BSA changes the dielectric constant of the reaction solution, and this is believed to be (at least partially) responsible for the agglutination enhancement. The proteolytic enzymes split off sialic acid-containing glycoproteins from the

Table 19–3. Common genes and gene combinations in Rh system.

	Genes		Expressed Antigens		Gene Frequency		
	Fisher-Race	Wiener	Fisher-Race	Wiener	Caucasian*	Black*	Chinese†
Rh-positive	*cDe*	R^0	c, D, e	Rh_o, hr′, hr″	0.026	0.29	0.03
	CDe	R^1	C, D, e	Rh_o, rh′, hr″	0.42	0.18	0.73
	cDE	R^2	c, D, E	Rh_o, rh″, hr′	0.14	0.11	0.19
	CDE	R^z	C, D, E	Rh_o, rh′, rh″	Rare	Rare	0.004
Rh-negative	*cde*	*r*	c, e	hr′, hr″	0.39	0.26	0.02
	Cde	*r′*	C, e	rh′, hr″	0.01	0.008	0.019
	cdE	*r″*	c, E	rh″, hr′	0.01	0.002	NI‡
	CdE	r^y	C, E	rh′, rh″	Rare	Rare	0.004

*From Giblett ER: *Genetic Markers in Human Blood.* Davis, 1969.

†From Mourant AE, Kopec AC, Domaniewska-Sobczak K: *The Distribution in the Human Blood Groups and Other Polymorphisms.* Oxford, 1976.

‡NI = Not included.

red cell membrane, resulting in reduction of the negative electric charge and weakening of repulsive forces, thus enabling IgG antibody molecules to bridge the cells. However, other mechanisms may be involved.

Prevention of Rh Immunization

In the early 1960s, American and British groups independently reported that Rh immunization could be prevented by passive administration of anti-D antibody in volunteers. Extensive clinical trials followed, and anti-D antibody has been used for more than a decade to prevent active immunization of Rh-negative mothers by Rh-positive fetal blood.

The mechanism of prevention is still somewhat unclear. The IgG anti-D may specifically inhibit the activation of a B cell response. Rapid clearance of the antigenic cells probably also plays an important role, since it has been known for a long time that mothers who have isoagglutinins against the fetus's cells (eg, mother group O, baby group A) do not form anti-D as readily as women with compatible blood groups. It was shown experimentally that passively administered anti-Kell could inhibit Rh immunization of Kell-negative Rh-negative subjects when challenged with Rh-positive Kell-positive erythrocytes.

A single dose of 150–300 μg of Rh immune globulin given within 72 hours after delivery covers the vast majority of cases. Some centers have introduced administration of anti-D antepartum to further increase the coverage.

Only a small proportion (3–7%) of mothers at risk would form anti-D antibodies even without administration of immune globulin. There are various reasons for failure to prevent sensitization, including a large volume of fetal blood released into the mother's circulation, immunization occurring during pregnancy before delivery, erroneous typing of the baby as Rh-negative, simply forgetting the injection, etc. Anti-D antibodies are not able to prevent a secondary response to D or an immune response to other blood group antigens.

If Rh-positive blood is transfused in error to an Rh-negative recipient, the patient is likely to make anti-D antibodies. If the recipient is a girl or a woman of fertile age, prevention of immunization may be tried if the error is found within 1 or 2 days. A suitable dose is about 4000 μg of anti-D per unit (450 mL) of transfused blood. This dose may be divided and given over a period of 2 days.

OTHER BLOOD GROUPS

There are some 20 blood group systems with more than 200 well-described antigens. Another 200-odd antigens have been reported, but they are very rare or have not been established as independent blood group antigens. In addition to ABO and Rh, only a few have clinical significance.

Kell System

The original Kell antigen has been given the symbol K, with k (sometimes called Cellano) as its allelic counterpart. K occurs in about 8% of the white population. There are a number of other antigens belonging to the Kell system (Kp^a, Kp^b, Js^a, Js^b, Ul^a, etc), but antibodies other than anti-K are rare. Anti-K is about as common as the Rh antibodies anti-c and anti-E, and it may cause hemolytic transfusion reactions and isoimmunization during pregnancy. The antibody, if present, is usually strong and easily detected by Coombs technique.

There is a rare variant in which all the Kell-related antigens are very weakly expressed on erythrocytes. This variant is associated with X-linked chronic granulomatous disease (see Chapter 20). The changes in Kell antigens probably reflect some basic failure in the structure of both red and white cell membranes.

Duffy System

About two-thirds of the white population have the antigen Fy^a. The antigen is destroyed upon conventional enzyme treatment, and the antibodies can be shown only with Coombs technique. Compared to other blood group antibodies, anti-Fy^a is a relatively common cause of hemolytic transfusion reactions. In the laboratory, the antibody is often weak and may be missed in compatibility testing.

The majority of blacks are of phenotype Fy(a−b−), which is extremely rare in whites; these people are also resistant to *Plasmodium vivax* malaria. The membrane structure that carries the polymorphic antigens of the Fy system is apparently a receptor for *P vivax*, and without it the parasite cannot invade the erythrocyte. In other forms of malaria, the same receptor is not involved, and Fy(a−b−) people are susceptible to other *Plasmodium* infections.

Other Systems

Antibodies to Kidd antigens Jk^a and Jk^b are not very common, but they activate complement, causing rapid hemolysis in vivo of incompatible red blood cells. Anti-Jk^a may be very weak in vitro, and the detection of this antibody is the main reason for the inclusion of anticomplement in Coombs reagents to test compatibility.

HLA antigens may sometimes be present on erythrocyte membrane, but they are always weak (see Chapter 6). The blood group antigens of Bg system have in fact been found to be HLA antigens, probably passively adsorbed onto red cell membrane. HLA antibodies do not destroy red cells even if they are positive with anti-Bg antisera.

Some complement components have polymorphic structures that may cause alloantibody formation in suitable subjects (see Chapter 10). The component C4 attaches on normal red cells to a variable degree, and the cells with sufficient C4 of an appropriate type are agglutinated by these antibodies. Anti-C4 alloantibodies, called anti-Rodgers and anti-Chido in blood group serology, do not usually cause clinical ill effects.

Cold agglutinins are common, but they are seldom strong enough to react at body temperature and conse-

quently to cause anemia in the patient (see Chapter 22). The specificity is usually anti-H, anti-I, anti-A_1, or a combination of these. They may cause problems in compatibility testing, but usually they are biologically insignificant. Moderately strong anti-I antibodies occur in connection with *Mycoplasma pneumoniae* infection, and anti-i antibodies are formed as a result of infectious mononucleosis. The antigen i occurs in fetal cells and is rendered I within the first 2 years of life. Rare adults have only i-antigen, and this property is inherited as a recessive trait.

COMPLICATIONS OF TRANSFUSIONS

Blood transfusion can be complicated by immunologic reaction to the contents of the transfused unit or contamination by infectious agents. Faulty transfusion technique can result in a variety of adverse reactions such as circulatory overload, air embolism, hypothermia, bleeding, and metabolic problems after massive transfusion. These reactions will not be discussed further.

Among the transfusion-associated fatalities, hemolysis was the predominant cause before the emergence of AIDS. Among the 70 fatal cases that were reported to United States Bureau of Biologics in 1976–1978, 44 patients died of acute hemolysis. Transfusion hepatitis was the second most important cause of death (10 cases). The rest of the reactions could not be attributed to blood transfusion, or they were caused by various isolated mishaps like bacterial contamination of the blood bag.

Immunologic Reactions

When careful cross-matching and antibody screening are done prior to transfusion, reactions from red cell incompatibility are rare. Serious reactions are usually due to clerical errors in labeling the test tubes or identifying the patient correctly. When a patient with a strong red cell antibody that activates complement by the classic pathway is transfused with incompatible blood (eg, ABO incompatibility), intravascular hemolysis occurs, often triggering disseminated intravascular coagulation. The severity of the hemolytic reaction depends on the strength of the antibody and the number of cells that have been transfused. The immediate symptoms include malaise, anxiety, backache, chest pain, and chills. In a severe reaction, the blood pressure may fall. The situation may be complicated by acute renal failure. The clinical signs can be masked by general anesthesia.

If the antibodies do not activate complement (eg, Rh and most other blood group antibodies), the transfused cells become coated by immunoglobulin and are captured by spleen macrophages via Fc receptors. The efficiency of this clearance depends on the number of antibody molecules on the red cells. Abundant anti-D antibodies may cause rapid extravascular destruction of the red cells, resulting in clinical symptoms, whereas weak antibodies against some other blood groups may cause a delayed transfusion reaction with shortened survival of the incompatible cells.

Reactions caused by antibodies against white blood cells are the most common immunologic complications of blood transfusion, occurring in 1–2% of all transfusions. Patients with a history of many transfusions or pregnancies are more prone to these reactions. Fortunately, the reactions are almost never severe. The main symptoms are fever and chills, but nausea and vomiting and hypotension sometimes occur. Before it is concluded that the reaction is caused by white cell antibodies, the possibility of a hemolytic reaction should be ruled out.

Class-specific or allospecific antibodies to IgA can cause severe anaphylactoid reactions, and these antibodies have been reported to occur in over 20% of individuals with IgA deficiency, which has a prevalence of about 1:800–1:400 in a healthy population (see Chapter 20). Nevertheless, these reactions are very rare in clinical practice. The reason for their scarcity is not yet known. There are some reports that antibodies to other plasma proteins may be responsible for occasional transfusion reactions, but these findings need confirmation.

Patients sometimes experience allergic reactions during or after the transfusion, commonly urticaria. The transfused blood may have contained traces of substances to which the recipient is hypersensitive, or the donor's allergy in form of reaginic IgE antibodies may have been temporarily transferred to the recipient. Studies to determine the cause of these reactions are usually unrewarding.

Treatment

If a transfusion reaction is suspected, the first step is to stop the transfusion. In hemolytic reactions, maintaining satisfactory kidney function is the most important part of therapy. Since the renal failure appears to be primarily due to ischemia, improvement of renal blood flow may be more useful than just increasing urinary output. Volume replacement with saline solutions, plasma fractions, or albumin is therefore essential. Diuretics such as furosemide should also be given. Hemodialysis or peritoneal dialysis may be needed. If the acute phase is successfully managed, the long-term prognosis is generally good.

Reactions due to leukocyte antibodies do not usually occur until a considerable amount of blood has been transfused, in contrast to hemolytic reactions, which may become obvious with small amounts of transfused blood. In mild fever-chill reactions, antipyretics often suffice. The transfusion may be continued if the possibility of a hemolytic reaction has been ruled out and if the blood is given at a reduced rate with careful observation of the patient, but usually it is better to transfuse leukocyte-poor blood.

Anaphylactoid reactions may require rapid measures to correct the fall of blood pressure and bronchospasm. Epinephrine is given subcutaneously or, in emergency cases, even intravenously. Corticosteroids may be useful, but otherwise the therapy is symptom-

atic. Reactions due to anti-IgA can occur after administration of only a few milliliters of IgA-containing plasma.

Investigation & Prevention

Laboratory investigations of a suspected hemolytic reaction are aimed at demonstrating hemolysis and antibodies incompatible with the transfused blood. In severe reactions, there is enough free hemoglobin to change the color of plasma and urine. The normal level of plasma hemoglobin is 20–30 mg/L; when it increases to approximately 200 mg/L, it will become visible to the naked eye. Hemoglobin is bound to haptoglobin, reducing its plasma levels to nearly zero. The breakdown product of heme, bilirubin, appears in plasma a few hours after the reaction. Determination of lactate dehydrogenase (LDH) may help in the diagnosis. In severe hemolysis, hemoglobin is bound to albumin to make methemalbumin, which has a characteristic dirty brownish color. Some of the laboratory investigations are listed in Table 19–4.

In serologic studies, the blood group of the patient and of the transfused blood unit are retyped, and the cross-match is repeated. Because the reaction may consume all of the antibodies, it is important to test both pre- and posttransfusion samples. The samples are examined for irregular antibodies, and a direct Coombs test is done on the posttransfusion sample.

The hemolytic reaction may be delayed if there were no antibodies at the time of transfusion or the antibodies were very weak and escaped detection. A delayed reaction is usually found by noticing fever and decreasing hemoglobin levels in the patient 3–21 days after transfusion. Determination of bilirubin and haptoglobin and performance of a direct Coombs test and antibody screening help in the diagnosis.

Serious hemolytic reactions are prevented if care is taken to properly identify the patient from whom the samples are drawn and to whom the blood is transfused. Test tubes should be adequately labeled and well-controlled methods used in blood grouping, compatibility testing, and screening for unexpected antibodies. Samples for blood grouping and cross-matching should be taken at different times, since faulty identification of the patient may lead to a severe reaction despite correct laboratory tests.

Table 19–4. Some laboratory investigations in suspected immunologic transfusion reaction.

Tests to detect hemolysis	
Color of plasma and urine	
Plasma and urine hemoglobin	
Plasma:	Bilirubin
	Haptoglobin
	LDH
	Methemalbumin
Tests to detect antibodies	
Retyping of patient's and donor's blood	
Repeating of cross-matches	
Antibody screening of patient's serum	
Direct antiglobulin on patient's cells	
Tests for cytotoxic white cell antibodies	
Determination of IgA level and tests for anti-IgA antibodies	

The practice of screening blood recipients for unexpected red cell antibodies (like anti-K, anti-Fy^a, etc) has increased the safety of transfusion and reduced the absolute need for a cross-match before transfusion. The blood banks should scrutinize transfusion practice in their own surgical services and derive a list of procedures that very seldom require blood. For such procedures, it is not necessary to hold cross-matched blood for individual patients. The patient is ABO- and Rh-typed, and if the antibody screening is negative, emergency release of ABO-compatible blood is permissible in the unlikely event that transfusion should become necessary. The cross-match is done afterward.

If the patient's temperature rises more than 1 °C during or immediately after the transfusion and there are no signs of hemolysis, the reaction is likely to be due to leukocyte antibodies. HLA antibodies can be screened with the cytotoxicity test (see Chapter 18). In some instances, the reaction is caused by granulocyte antibodies, and the cytotoxicity test then employed on lymphocytes may be negative. The agglutination tests for demonstration of antigranulocyte antibodies are not always reliable and are difficult to standardize.

Febrile reactions can be eliminated or at least made less severe by using leukocyte-poor blood. Theoretically, these patients should receive HLA-matched blood, but because of the large number of different HLA antigens, this is usually not feasible even in well-equipped blood banks with tissue typing laboratories.

Patients with anaphylactoid reactions should be investigated for the presence of IgA and possible anti-IgA antibodies. Immunodiffusion is not sensitive enough to determine lack of IgA, but more sensitive methods such as hemagglutination inhibition, radioimmunoassay, or enzyme immunoassay should be used. Anti-IgA antibodies can be measured by passive hemagglutination if isolated IgA myeloma proteins are available for the assay. A patient with class-specific anti-IgA should be transfused with either IgA-deficient blood or with thoroughly washed red cell concentrate.

Changing Group of Transfused Blood

As a rule, only blood of the patient's own ABO and Rh group should be given. In emergency cases, or if the blood bank cannot provide a particular blood type, ABO-dissimilar red cell concentrate can be given if the cross-match is negative. Whole blood should not be given, since it contains antibodies that react with the patient's own red cells. Even in emergencies, the transfusion can often be started with infusion of salt solutions and plasma substitutes, and meanwhile the patient's blood group can be typed. If blood of the patient's group is not available, group O red cell concentrate should be given to A and B persons, whereas AB people can be transfused either with A or B red cell concentrate. A is to be preferred, since it is more widely available.

Rh-negative blood may be given to Rh-positive re-

cipients, whereas Rh-positive blood causes anti-D antibody formation in Rh-negative patients. This immunization does not cause immediate ill effects (provided the patient does not already have Rh antibodies), but synthesized antibodies shorten the survival time of the transfused cells. The age of the patient, acute and future needs for blood, and the prognosis of the disease should be considered before deciding to change from Rh-negative to Rh-positive blood. Rh-negative girls and women of fertile age should not be transfused with Rh-positive blood.

INFECTIONS

The most common disease transmitted by blood transfusion is hepatitis, which may be hepatitis B surface antigen (HBsAg)-positive or -negative. In the latter case, it is called non-A, non-B hepatitis (see Chapter 25). Hepatitis A is transmitted extremely rarely by blood. The incidence of posttransfusion hepatitis varies greatly with different blood donor populations and is generally higher when donors are paid. Screening of blood units for HBsAg has greatly reduced the incidence of hepatitis B, so that the great majority of posttransfusion hepatitis cases are of type non-A, non-B.

The incubation time for posttransfusion hepatitis is highly variable, but it is generally agreed that it is not shorter than 2 weeks and not longer than 6 months. Since serologic tests are not yet available for non-A, non-B hepatitis, the cause-effect relationship is not always clear-cut. Other causes of hepatic dysfunction must be excluded before the diagnosis of posttransfusion hepatitis is established.

All blood units must be tested for absence of HBsAg. Only so-called third-generation tests (based on radioimmunoassay, enzyme immunoassay, or reversed passive hemagglutination) are acceptable, since the less sensitive methods miss some positive samples. Prophylaxis with anti-HBsAg hyperimmune globulin is recommended in connection with accidental exposure to HBsAg-positive blood (needle scratches, minor wounds, etc), but it is not effective if a whole unit of HBsAg-positive blood has been erroneously transfused to the patient. Now that safe hepatitis B vaccines are available, selected groups such as laboratory personnel can be protected by active immunization.

AIDS is a serious new risk of blood transfusion. The causative agent was identified at about the same time in Paris and in Washington, DC, and was given the names lymphadenopathy virus (LAV) and human T-lymphotropic virus type III (HTLV-III), respectively. A test to measure anti-HTLV-III antibodies by enzyme immunoassay (ELISA) was quickly developed, and systematic screening of blood donors was started in the USA and in other countries in the first half of 1985. Since the virus may circulate in the blood in the absence of antibodies, it is important that high-risk population groups (homosexual and bisexual men, drug addicts, etc) refrain from blood donation even if the blood units are tested for anti-HTLV-III. Despite screening, transfusion-associated AIDS cases infected earlier will continue to appear for some years, since the incubation period may be 5 years on average.

The AIDS issue is an emotional one. Informing the blood donor of a positive anti-HTLV-III result remains difficult; maintenance of confidentiality is of the utmost importance. The donor should not be informed of a positive result before an appropriate confirmatory test (such as the Western blot test) shows that the reaction is not falsely positive.

Available data suggest that AIDS is not particularly contagious. If precautions similar to those for HBsAg-positive samples are followed, the risk of laboratory personnel acquiring HTLV-III infection seems minimal.

Screening for syphilis antibodies is also required before blood is transfused. However, blood stored for 48–72 hours does not transmit syphilis, and the usefulness of screening the blood donors in a modern society has been questioned.

Cytomegalovirus (CMV) was implicated a long time ago in the development of the so-called postperfusion syndrome after open heart surgery when the patient had received multiple units of fresh blood. CMV disease is relatively mild in adults with normal immune function, but in immunocompromised patients it can be very severe. As many as 20% of patients receiving allogeneic bone marrow transplants as treatment for leukemia die from CMV pneumonia. Since CMV is latent in leukocytes of persons with CMV antibodies, many centers have started transfusion of anti-CMV-negative blood to selected patient groups. CMV immune globulin probably reduces the risk of severe disease, and its use may be an alternative means to prevent this complication of transfusion.

Malaria and some other infectious diseases may be transmitted via blood transfusion. The risk of these diseases is increasing with more tourists visiting endemic areas. Visitors to these places are usually not permitted to donate blood for at least 6 months. Some centers have access to tests for *Plasmodium* antibodies. The results are helpful when the final decision is made whether or not the donor can give blood for normal transfusion purposes. Donors with a history of malaria are rejected permanently or at least for several years.

Occasionally, the blood unit may be contaminated by microbes that grow in the cold. Transfused bacterial endotoxin can trigger shock and disseminated intravascular coagulation, with high mortality rates.

BLOOD COMPONENT THERAPY

In modern transfusion practice, the different cellular and plasma components of blood should be used selectively. Blood component therapy results in fewer complications and optimal utilization of blood. Since

there is a worldwide shortage of available blood, rational use of blood components should be greatly encouraged.

Packed Red Blood Cells (Red Cell Concentrates)

Packed red blood cells are indicated for the treatment of most anemias where transfusion is necessary, except for anemia associated with hypovolemic shock. This includes all chronic anemias, subacute and chronic blood loss, and surgical patients who require less than 2 or 3 units of blood replacement at surgery. Compared to whole blood, packed red blood cells have the following advantages: (1) ability to process whole blood more efficiently to obtain other valuable blood components from the same unit; (2) more oxygen-carrying capacity with less risk of circulatory overload; and (3) less infusion of sodium, potassium, ammonia, lactate, citrate, and donor antibodies.

Leukocyte-poor red blood cells are packed cells with the buffy coat containing most of the leukocytes and platelets removed. They are indicated for treatment of patients who have febrile transfusion reactions caused by leukocyte or platelet antibodies. It is not possible to make the blood units completely free of leukocyte antigens, but the amount can be greatly reduced by centrifugation and filtration.

Frozen red cells are prepared from packed red cells by freezing them in a cryoprotective agent and then thawing and washing them prior to transfusion. Frozen red cells are currently indicated for transfusion of patients with rare blood types when blood is not available in any other form; for patients with leukocyte or platelet antibodies so potent that they react to leukocyte-poor red blood cells; and for patients who need washed red cells because they react to plasma proteins.

Platelet Concentrates

Platelets can be separated from whole blood by differential centrifugation. They may be given to stop or prevent bleeding in thrombocytopenic patients with aplastic anemia or acute leukemia. They may also be given following massive transfusion of stored blood, but they have only limited usefulness in ITP, DIC, and other thrombocytopenias due to increased platelet destruction. In patients with antibodies to histocompatibility antigens, HLA-matched platelets may be given, but this requires a well-equipped blood bank with a tissue typing laboratory and a large selection of HLA-typed donors.

White Cell Concentrate

Large amounts of white cells could be useful and perhaps lifesaving in certain granulocytopenic patients with serious septic infections. It has been shown, however, that the number of cells needed for significant clinical results is so high that it is doubtful whether the currently available white cell concentrates are useful. Their popularity has declined during recent years.

Coagulation Factors

When fresh plasma is rapidly frozen and then thawed, the resulting cryoprecipitate contains 30–50% of the factor VIII activity of the plasma. In addition, there is a fair amount of fibrinogen and fibronectin. Cryoprecipitate is used for treatment of hemophilia A and von Willebrand's disease and can also be used when fibrinogen is needed. Factor VIII can be further purified and concentrated. Factor VIII concentrates are especially suitable for home therapy, but because they are prepared from large plasma pools, the risk of transmitting infectious diseases is higher. The concentrates are therefore heat-treated to inactivate the HTLV-III virus and reduce the possibility of hepatitis transmission. Factor II, VII, IX, and X concentrates are also available for the rare patients who lack these coagulation factors.

Other Plasma Proteins

Albumin is the most widely used plasma derivative. It is prepared by Cohn's cold ethanol fractionation procedure. Plasma protein fraction (PPF) contains small amounts of other proteins in addition to albumin. The different albumin preparations are used as plasma substitutes for blood volume expansion. Their main advantage over plasma is the avoidance of transmission of hepatitis and the lack of blood group antibodies. They are useless for parenteral nutrition.

The immunoglobulins are also separated in the fractionation process and after concentration may be used as antibody source for passive immunization against some infectious diseases. Immunodeficient patients with hypogammaglobulinemia may need regular immunoglobulin injections as prophylactic therapy (see Chapter 20). Immunoglobulin prepared from Rh-immunized mothers or male volunteers is widely used for prevention of hemolytic disease of the newborn. Immunoglobulins can also be prepared so that they may be given in large doses intravenously. Trials with such preparations have yielded promising results in the treatment of some autoimmune diseases as well as antibody deficiency diseases.

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Section III. Clinical Immunology

Immunodeficiency Diseases

20

Arthur J. Ammann, MD

Four major immune systems assist the individual in the defense against a constant assault by viral, bacterial, fungal, protozoal, and nonreplicating agents that have the potential of producing infection and disease. These systems consist of antibody-mediated (B cell) immunity, cell-mediated (T cell) immunity, phagocytosis, and complement. Each system may act independently or in concert with one or more of the others.

Deficiency of one or more of these systems may be congenital (eg, X-linked infantile hypogammaglobulinemia) or acquired (eg, acquired hypogammaglobulinemia). Deficiencies of the immune system may be secondary to an embryologic abnormality (eg, the DiGeorge syndrome), may be due to an enzymatic defect (eg, chronic granulomatous disease), or may be of unknown cause (eg, Wiskott-Aldrich syndrome).

In general, the symptomatology of immunodeficiency is related to the degree of deficiency and the particular system that is deficient in function. General features are listed in Table 20–1 parts A and B. Features associated with specific immunodeficiency disorders are listed in Table 20–1 part C. The types of infections that occur often provide an important clue to the type of immunodeficiency disease present. Recurrent bacterial otitis media and pneumonia are common in hypogammaglobulinemia. Patients with defective cell-mediated immunity are susceptible to infection with fungal, protozoal, and viral organisms that may present as pneumonia or chronic infection of the skin and mucous membranes or other organs. Systemic infection with uncommon bacterial organisms, normally of low virulence, is characteristic of chronic granulomatous disease. Other phagocytic disorders are associated with superficial skin infections or systemic infections with pyogenic organisms. Complement deficiencies are associated with recurrent infections with pyogenic organisms.

Numerous advances have recently been made in the diagnosis of specific immunodeficiency disorders (Table 20–2). Screening tests are available for each component of the immune system (Table 20–3). These tests enable the physician to diagnose over 75% of immunodeficiency disorders. The remainder can be diagnosed by means of more complicated studies that may not be available in all hospital laboratories.

In addition to antimicrobial agents for treatment of specific infections, new forms of immunotherapy are available to assist in the control of immunodeficiency or perhaps even to cure the underlying disease (Table 20–4). The usefulness of some of these treatment methods, such as bone marrow transplantation, may be limited by the availability of suitable donors. The discovery of enzyme deficiencies (eg, adenosine deaminase) in association with immunodeficiency offers a potential new avenue of therapy.

Table 20–1. Clinical features associated with immunodeficiency.*

A. Features frequently present and highly suspicious:	
1. Chronic infection	
2. Recurrent infection (more than expected)	
3. Unusual infecting agents	
4. Incomplete clearing between episodes of infection or incomplete response to treatment	
B. Features frequently present and moderately suspicious:	
1. Skin rash (eczema, *Candida*, etc)	
2. Diarrhea (chronic)	
3. Growth failure	
4. Hepatosplenomegaly	
5. Recurrent abscesses	
6. Recurrent osteomyelitis	
7. Evidence of autoimmunity	
C. Features associated with specific immunodeficiency disorders:	
1. Ataxia	
2. Telangiectasia	
3. Short-limbed dwarfism	
4. Cartilage-hair hypoplasia	
5. Idiopathic endocrinopathy	
6. Partial albinism	
7. Thrombocytopenia	
8. Eczema	
9. Tetany	

*Reproduced, with permission, from Ammann A, Wara D: Evaluation of infants and children with recurrent infections. *Curr Probl Pediatr* 1975;5:3. [Entire issue.]

Immunodeficiency disorders are discussed below under 4 main categories: antibody (B cell) deficiency, cellular (T cell) deficiency, phagocytic dysfunction, and complement deficiency. In general, the terminology used for specific deficiencies agrees with the classification recently proposed by a committee of the World Health Organization (Table 20–2).

ANTIBODY (B CELL) IMMUNODEFICIENCY DISORDERS

Antibody immunodeficiency disorders comprise a spectrum of diseases with decreased immunoglobulins

Table 20–2. Classification of immunodeficiency disorders.

I. **Antibody (B cell) immunodeficiency diseases**
- X-linked hypogammaglobulinemia (congenital hypogammaglobulinemia)
- Transient hypogammaglobulinemia of infancy
- Common, variable, unclassifiable immunodeficiency (acquired hypogammaglobulinemia)
- Immunodeficiency with hyper-IgM
- Selective IgA deficiency
- Selective IgM deficiency
- Selective deficiency of IgG subclasses
- Secondary B cell immunodeficiency associated with drugs, protein-losing states
- B cell immunodeficiency associated with 5′-nucleotidase deficiency
- X-linked lymphoproliferative disease

II. **Cellular (T cell) immunodeficiency diseases**
- Congenital thymic aplasia (DiGeorge syndrome)
- Chronic mucocutaneous candidiasis (with or without endocrinopathy)
- T cell deficiency associated with purine nucleoside phosphorylase deficiency
- T cell deficiency associated with absent membrane glycoprotein
- T cell deficiency associated with absent class I MHC antigens

III. **Combined antibody-mediated (B cell) and cell-mediated (T cell) immunodeficiency diseases**
- Severe combined immunodeficiency disease (autosomal recessive, X-linked, sporadic)
- Cellular immunodeficiency with abnormal immunoglobulin synthesis (Nezelof's syndrome)
- Immunodeficiency with ataxia-telangiectasia
- Immunodeficiency with eczema and thrombocytopenia (Wiskott-Aldrich syndrome)
- Immunodeficiency with thymoma
- Immunodeficiency with short-limbed dwarfism
- Immunodeficiency with adenosine deaminase deficiency
- Episodic lymphopenia with lymphotoxin
- GVH disease
- Acquired immunodeficiency syndrome (AIDS)

IV. **Phagocytic dysfunction**
- Chronic granulomatous disease
- Glucose-6-phosphate dehydrogenase deficiency
- Myeloperoxidase deficiency
- Chédiak-Higashi syndrome
- Job's syndrome
- Tuftsin deficiency
- "Lazy leukocyte syndrome"
- Elevated IgE, defective chemotaxis, eczema, and recurrent infections

V. **Complement abnormalities and immunodeficiency diseases**
- C1q, C1r, and C1s deficiency
- C2 deficiency
- C3 deficiency (type I, type II)
- C4 deficiency
- C5 dysfunction, C5 deficiency
- C6 deficiency
- C7 deficiency
- C8 deficiency
- C9 deficiency

Table 20–3. Initial screening evaluation.

Antibody-mediated immunity	
	Quantitative immunoglobulin levels: IgG, IgM, IgA
	Schick test: measures specific IgG antibody response
	Isohemagglutinin titer (anti-A and anti-B): measures IgM function
Cell-mediated immunity	
	White blood count with differential: measures total lymphocytes
	Delayed hypersensitivity skin tests: measures specific T cell and macrophage response to antigens
Phagocytosis	
	White blood count with differential: measures total neutrophils
	NBT, chemiluminescence: measure neutrophil metabolic function
Complement	
	Hemolytic complement quantitation (CH_{50}): quantitates complement activity
	C3 level: measures amount of important complement component

ranging from complete absence of all classes to selective deficiency of a single immunoglobulin class. The degree of symptoms found in patients with antibody immunodeficiency disorders is chiefly dependent on the degree of antibody deficiency. Patients with hypogammaglobulinemia become symptomatic earlier and experience more severe disease than patients with selective immunoglobulin deficiency. Screening tests for the specific diagnosis of antibody deficiency disorders are readily available in most hospital laboratories (see Table 20–3 and Chapter 17) and usually afford early diagnosis with prompt institution of appropriate treatment. Newer procedures such as counting B cells in peripheral blood, determination of in vitro immunoglobulin production, and suppressor cell assays permit more precise diagnosis as well as a greater understanding of the causes (Table 20–5).

X-LINKED INFANTILE HYPOGAMMAGLOBULINEMIA

Major Immunologic Features

- Symptoms of recurrent pyogenic infections usually begin by 5–6 months of age.
- IgG less than 200 mg/dL with absence of IgM, IgA, IgD, and IgE.
- Absence of B cells in peripheral blood.
- Patients respond well to treatment with γ-globulin.

General Considerations

In 1952, Bruton described a male child with hypogammaglobulinemia, and this is now recognized as the first clinical description of an immunodeficiency disorder. The disorder is easily diagnosed using standard laboratory tests that demonstrate marked deficiency or complete absence of all 5 immunoglobulin

Table 20–4. Treatment of immunodeficiency.

Treatment	B Cell Disorders	T Cell Disorders	Phagocytic Disorders	Complement Disorders
γ-Globulin.	X-linked hypogammaglobulinemia. Acquired hypogammaglobulinemia. Secondary hypogammaglobulinemia when associated with infection. Do not use in selective IgA deficiency.	Use only when absent antibody response is demonstrated. Not recommended for intramuscular use in Wiskott-Aldrich syndrome. New intravenous preparations may be used. γ-Globulin does not transmit virus, eg, hepatitis, retrovirus.	Not recommended.	Not recommended.
Hyperimmune gamma globulin.	Use in above disorders when specific exposure has occurred.	May be used when specific exposure has occurred.	May be used when specific exposure has occurred.	May be used when specific exposure has occurred.
Frozen plasma by intravenous infusion. Largely replaced by intravenous γ-globulin.	X-linked hypogammaglobulinemia and acquired hypogammaglobulinemia when intramuscular administration is not tolerated or is ineffective.	Use only when absent antibody response is demonstrated. Irradiate to prevent GVH disease.	Not recommended.	Use with caution. Plasma may exacerbate autoimmune disease.
Infusions of white cells.	Not recommended.	Not recommended.	Questionable value.	Not recommended.
Infusions of red cells.	Not recommended.	May be of benefit in certain enzyme deficiencies associated with immunodeficiency (adenosine deaminase, purine nucleoside phosphorylase). Irradiate to prevent GVH.	Not recommended.	Not recommended.
Bone marrow transplant.	Not recommended.	Use only when impaired T cell function is present. Histocompatible donor preferred.	Not recommended.	Not recommended.
Fetal thymus transplantation.	Not recommended.	DiGeorge syndrome. Selective use in other combined immunodeficiency disorders.	Not recommended.	Not recommended.
Cultured thymus epithelium.	Not recommended.	Selective cases of T cell disorders where no suitable bone marrow donor is available.	Not recommended.	Not recommended.
Fetal liver transplantation.	Not recommended	Used in past in severe combined immunodeficiency in absence of suitable bone marrow donor. Rarely used now.	Not recommended.	Not recommended.
Transfer factor.	Not recommended.	May be successful in chronic candidiasis when combined with antifungal agent. Highly debatable effect in other disorders.	Not recommended.	Not recommended.
Thymosin, TP1, α_1, facteur thymique sérique.	Not recommended.	Limited evaluation to date. May enhance T cell function in a variety of T cell disorders, including DiGeorge syndrome. No effect in chronic candidiasis or severe combined immunodeficiency.	Not recommended.	Not recommended.

classes. Male infants with this disorder usually become symptomatic following the natural decay of transplacentally acquired maternal immunoglobulin at about 5–6 months of age. They suffer from severe chronic bacterial infections that can be controlled readily with γ-globulin and antibiotic treatment. The incidence of this disorder in the USA is not precisely known, but estimates in the United Kingdom suggest an overall incidence of one case per 100,000 population. Recently, 2 female siblings with congenital hypogammaglobulinemia have been reported.

Immunologic Pathogenesis

Extirpation of the bursa of Fabricius in avian species results in complete hypogammaglobulinemia. Several investigators think that the bursa equivalent in humans consists of gastrointestinal tract-associated lymphoid tissue, ie, tonsils, adenoids, Peyer's patches, and appendix. Other investigators consider that the bursa equivalent in humans exists in the bone marrow, which provides a source of stem B cells. In X-linked infantile hypogammaglobulinemia, it is felt that this stem cell population is absent, resulting in complete absence of plasma cells and peripheral blood B lymphocytes. Recent investigations have provided evidence of pre-B cells in the marrow and peripheral blood of patients. These pre-B cells fail to secrete immunoglobulin.

Table 20–5. Evaluation of antibody-mediated immunity.

Test	Comment
Protein electrophoresis	Use to presumptively diagnose hypogammaglobulinemia or to evaluate for paraproteins.
Radial immunodiffusion	Best procedure for quantitation of IgG, IgM, IgA, and IgD.
Radioimmunoassay	IgE quantitation.
Schick test	DTP immunization must be complete. Use to evaluate IgG function.
Isohemagglutinins	Use to evaluate IgM function. Titer > 1:4 after 1 year of age.
Specific antibody response	Use to evaluate immunoglobulin function. Tetanus, diphtheria, typhoid, etc. Do not immunize with live virus if immunodeficiency is suspected.
B cell quantitation	Measurement of the number of circulating B cells. Normal is 10–25% (total IgG-, IgM-, IgD-, and IgA-bearing cells).
In vitro immunoglobulin synthesis	Determines helper/suppressor T cell function in hypogammaglobulinemia.

Clinical Features

A. Symptoms and Signs: Patients with X-linked infantile hypogammaglobulinemia usually remain asymptomatic until 5–6 months of age, when passively transferred maternal IgG reaches its lowest level. The loss of maternal immunoglobulin usually coincides with the age at which these children are increasingly exposed to pathogens. Initial symptoms consist of recurrent bacterial otitis media, bronchitis, pneumonia, meningitis, dermatitis, and occasionally arthritis or malabsorption. Many infections respond promptly to antibiotic therapy, and this response occasionally will delay the diagnosis of hypogammaglobulinemia. The most common organisms responsible for infection are *Streptococcus pneumoniae* and *Haemophilus influenzae;* other organisms such as streptococci and certain gram-negative bacteria are occasionally responsible. Although patients normally have intact cell-mediated immunity and respond normally to viral infections such as varicella and measles, there have been reports of paralytic poliomyelitis and progressive encephalitis following immunization with live virus vaccines or exposure to wild virus. Fatal echovirus infection has been reported in patients with congenital hypogammaglobulinemia. The encephalitis in a few patients has responded to treatment with intravenous γ-globulin or plasma. A relationship of echovirus infection, dermatomyositis, and hypogammaglobulinemia has been proposed. These observations suggest that some patients with hypogammaglobulinemia may also be unusually susceptible to some viral illnesses.

An important clue to the diagnosis of hypogammaglobulinemia is the failure of infections to respond completely or promptly to appropriate antibiotic therapy. In addition, many patients with hypogammaglobulinemia have a history of continuous illness; ie, they do not have periods of well-being between bouts of illness.

Occasionally, patients with hypogammaglobulinemia may not become symptomatic until early childhood. Some of these patients may present with other complaints, such as chronic conjunctivitis, abnormal dental decay, or malabsorption. The malabsorption may be severe and may cause retardation of both height and weight. Frequently the malabsorption is associated with infestation by *Giardia lamblia*. A disease resembling rheumatoid arthritis has been reported in association with hypogammaglobulinemia. This occurs principally in untreated infants or is an indication for more intensive therapy with γ-globulin.

Physical findings usually relate to recurrent pyogenic infections. Chronic otitis media and externa, serous otitis, conjunctivitis, abnormal dental decay, and eczematoid skin infections are frequently present. Despite the repeated infections, lymphadenopathy and splenomegaly are absent.

B. Laboratory Findings: The diagnosis of infantile X-linked hypogammaglobulinemia is based on the demonstration of absence or marked deficiency of all 5 immunoglobulin classes. Although a diagnosis can be established utilizing immunoelectrophoresis, specific quantitation of immunoglobulins is recommended, especially during early infancy. Total immunoglobulins are usually less than 250 mg/dL. IgG is usually less than 200 mg/dL, while IgM, IgA, IgD, and IgE are extremely low or absent. Rarely, patients have complete hypogammaglobulinemia except for the presence of normal amounts of IgE. It is unusual for patients with hypogammaglobulinemia to have depressed levels of IgG and normal levels of IgM or IgA. Before a diagnosis of hypogammaglobulinemia is established, there should be a demonstration of failure to make antibody following antigenic stimulation. The diagnosis may be difficult in infants under 6 months of age because of maternal IgG in the serum.

Isohemagglutinins that result from natural immunization are present in normal infants of the appropriate blood group by 1 year of age. Titers of anti-A and anti-B should be greater than 1:4 in normal individuals. Individuals who have received the complete series of DTP immunizations should react negatively to the Schick test. Previous γ-globulin therapy (within 1 month) may result in a nonreactive Schick test in a patient with hypogammaglobulinemia. Antibody to a specific antigen may be measured following immunization, but a patient suspected of having an immunodeficiency disorder should never be immunized with live attenuated viral vaccine. Although lymph node biopsies have been recommended in the past, with currently available diagnostic studies this would appear to be an unnecessary procedure. Rarely, in difficult cases, an intestinal biopsy to determine if plasma cells are present may be necessary to assist in the diagnosis. In X-linked infantile hypogammaglobulinemia, there is a complete absence of plasma cells in the lamina propria of the gut. Studies of peripheral blood lymphocytes indicate a complete absence of cir-

culating B cells, with normal to increased numbers of T cells. Studies of T cell immunity indicate that this system is intact. Delayed hypersensitivity skin tests are usually positive; isolated peripheral blood lymphocytes respond normally to PHA and allogeneic cells (MLC); and there are normal numbers of circulating peripheral blood T cells.

C. Other Studies: X-ray of the lateral nasopharynx has been suggested as a method of demonstrating the lack of lymphoid tissue. It is doubtful that this information adds significantly to the findings on physical examination. X-rays of the sinuses and chest should be obtained at regular intervals to follow the course of the patient and to determine adequacy of treatment. Pulmonary function studies should also be performed on a regular basis, beginning as soon as patient cooperation can be obtained. Patients with hypogammaglobulinemia who have gastrointestinal tract symptoms should be investigated for the presence of *G lamblia* and other causes of malabsorption.

Immunologic Diagnosis

Total immunoglobulins are less than 250 mg/dL; IgG is less than 200 mg/dL; and IgM, IgA, IgD, and IgE are markedly reduced or absent. B cells are absent in peripheral blood, and there are no plasma cells containing immunoglobulins in tissue and lymph nodes. Lymph nodes are markedly depleted in B cell-dependent areas. No antibodies are formed following specific immunization. T cell immunity is intact. NK cell activity is normal.

Differential Diagnosis

A diagnosis of X-linked infantile hypogammaglobulinemia may be difficult to establish in the age range of 5–9 months. By this time most infants have lost their maternal immunoglobulins and are susceptible to recurrent infections. The majority of normal infants during this time have levels of IgG less than 350 mg/dL but usually show some evidence of IgM and IgA production (usually > 20 mg/dL). If the diagnosis appears uncertain, several approaches may be utilized. Immunoglobulin levels may be determined again 3 months after the initial values. If there is an increase in IgG, IgM, or IgA, it is highly unlikely that the patient has hypogammaglobulinemia. Alternatively, the patient may be immunized with killed vaccines and specific antibody levels determined. The most difficult diagnostic problem is the differentiation of prolonged physiologic hypogammaglobulinemia from X-linked infantile hypogammaglobulinemia. In the former instance, the hypogammaglobulinemia may be severe enough to require treatment, and immunoglobulin levels may be identical to those of patients with congenital hypogammaglobulinemia. Normal production of immunoglobulins may not occur until as late as 18 months of age in patients with physiologic hypogammaglobulinemia. However, in most instances, these patients will begin to produce their own immunoglobulin despite concurrent γ-globulin administration. This is manifested by increasing levels of IgG as well as IgM and IgA. Since commercial γ-globulin contains less than 10% of IgM or IgA, a gradual increase in these values argues strongly against congenital hypogammaglobulinemia. The best way to avoid mistaking congenital hypogammaglobulinemia for prolonged physiologic hypogammaglobulinemia in infants is to carefully compare immunoglobulin levels with age-matched controls (see Chapter 17) and to obtain sequential measurements of immunoglobulins at 3-month intervals during the first year of diagnostic uncertainty.

Patients with severe malabsorption—particularly protein-losing enteropathy—may have severely depressed levels of immunoglobulins. In most instances, a diagnosis of protein-losing enteropathy can be established by the demonstration of a concomitant deficiency of serum albumin. Occasionally, however, patients with severe malabsorption and primary hypogammaglobulinemia may also lose albumin through the intestinal tract. Under these circumstances, a diagnosis can best be made by obtaining an intestinal biopsy. Patients with protein-losing enteropathy have normal numbers of plasma cells in the gut that contain intracellular immunoglobulins.

Polyarthritis may be a presenting feature in patients with hypogammaglobulinemia. Most patients with juvenile rheumatoid arthritis have elevated levels of immunoglobulins. Patients with arthritis and hypogammaglobulinemia usually respond promptly following institution of γ-globulin therapy.

Patients with chronic lung disease should also be suspected of having cystic fibrosis, asthma, α_1-antitrypsin deficiency, or immotile cilia syndrome.

Treatment

Treatment schedules have varied. Commercial γ-globulin is the mainstay of therapy and is usually given in starting doses of 0.2–0.4 mL/kg as a single intramuscular dose. The final amount given and the frequency of injections should be regulated by the control of symptoms rather than a calculated amount or a particular serum level, since metabolism of IgG varies in different individuals. Following the administration of an initial dose once a month, the amount given can be increased by 2–3 mL per injection. If the amount injected into a single site becomes too large, it may be divided and given in 2 sites at the same time. As amounts continue to increase, injections may be given every 2 weeks or every week. Some patients prefer weekly injections of smaller amounts rather than large monthly injections.

Anaphylactoid reactions to γ-globulin administration have been observed. These are not mediated through the classic IgE allergic pathway, since most patients with hypogammaglobulinemia lack IgE. The chief causes of these reactions are aggregate formation in the γ-globulin preparation and inadvertent intravenous administration. Patients who have repeated reactions to γ-globulin should be treated with an alternative preparation obtained from a different commercial source. If patients continue to have reactions, it may

be necessary to centrifuge the preparation to remove aggregates prior to administration. Intravenous γ-globulin is now licensed for routine clinical use. Appropriate clinical application would be in patients who fail to respond to intramuscular γ-globulin or who might benefit from large amounts of antibody. The dose of intravenous γ-globulin is 100–400 mg/kg given over 2–4 hours. In very sick patients, it can be given weekly or daily. In contrast to intramuscular γ-globulin, intravenous preparations result in significant increases of serum IgG. γ-Globulin is not capable of transmitting virus, eg, hepatitis virus, retrovirus.

Additional therapy may be necessary in patients who fail to respond to maximum doses of γ-globulin. Because commercial γ-globulin contains primarily IgG and little IgM or IgA, some immunologists consider that there is a beneficial effect of monthly intravenous infusions of fresh-frozen plasma. The dose of plasma used is 10 mL/kg. In adult patients, 1–2 units may be used. To avoid the risk of hepatitis, a "buddy system" has been devised whereby the patient receives plasma from a single reliable donor. Alternatively, screening of plasma for HBsAg should be done. γ-Globulin therapy should be continued if frozen plasma is used. Although fresh-frozen plasma therapy may provide other antibacterial substances in addition to IgG, IgM, and IgA, its use has been largely replaced by intravenous γ-globulin.

Continuous use of antibiotics may be necessary. Broad-spectrum antibiotics such as ampicillin in low to moderate doses may be effective in controlling recurrent infection. Physical therapy with postural drainage should be utilized in patients with chronic lung disease or bronchiectasis.

Occasionally, a patient with hypogammaglobulinemia may be discovered who has no or minimal symptoms. These patients should receive γ-globulin therapy even though they have not experienced repeated infection. Avoidance of infection with subsequent permanent complications is an important part of treatment in these patients.

Malabsorption, which is occasionally found in patients with hypogammaglobulinemia, usually responds to treatment with γ-globulin or intravenous fresh-frozen plasma (or both). If *Giardia* is found, it should be treated with metronidazole in doses of 35–50 mg/kg/24 h in 3 divided doses for 10 days in children and 750 mg orally 3 times a day for 10 days in adults.

Complications & Prognosis

Although patients with congenital hypogammaglobulinemia have survived to the second and third decades, the prognosis must be guarded. Despite what may appear to be adequate γ-globulin replacement therapy, many patients develop chronic lung disease. The presence of severe infection early in infancy may result in irreversible damage. Patients who recover from meningitis may have severe neurologic handicaps. Patients with severe pulmonary infection frequently develop bronchiectasis and chronic lung disease. Regular examinations and prompt institution of therapy are necessary to control infections and prevent complications. Fatal echovirus infections of the central nervous system have been reported even in patients receiving γ-globulin therapy. Some of these infections have been associated with dermatomyositis. Some patients may develop leukemia or lymphoma.

TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY

Almost all infants go through a period of hypogammaglobulinemia at approximately 5–6 months of age. Under normal circumstances, maternal IgG is passively transferred to the infant beginning at the 16th week of gestational life. At the time of birth the serum IgG value of the infant is usually higher than that of the mother. IgA, IgM, IgD, and IgE are not placentally transferred under normal circumstances. In fact, the presence of elevated values of IgM or IgA in cord blood suggests premature antibody synthesis, usually as a result of intrauterine infection. Over the first 4–5 months of life, there is a gradual decrease in serum IgG and a gradual increase of serum IgM and IgA. The IgM usually rises more rapidly than the IgA. At 5–6 months, serum IgG reaches its lowest level (approximately 350 mg/dL). At this point, many normal infants begin to experience recurrent respiratory tract infections. Immunoglobulin values obtained at this age must be compared to those of normal infants of the same age. Occasionally, an infant may fail to produce normal amounts of IgG at this time, resulting in transient hypogammaglobulinemia, or so-called physiologic hypogammaglobulinemia. The presence of normal serum levels of IgM or IgA argues strongly against a diagnosis of X-linked hypogammaglobulinemia. However, some infants with transient hypogammaglobulinemia may also fail to produce normal amounts of IgM or IgA.

Additional studies may be of no diagnostic usefulness, since many infants fail to respond to immunization at this age and isohemagglutinin titers may be low. Under these circumstances, a lymph node or intestinal biopsy may assist in establishing the diagnosis. Patients with congenital hypogammaglobulinemia lack plasma cells containing immunoglobulins in the intestinal tract and in peripheral lymph nodes. In addition, patients with congenital hypogammaglobulinemia lack circulating peripheral blood B cells. If the patient is not experiencing severe recurrent infection, it is best to wait 3–5 months and repeat immunoglobulin measurements rather than perform invasive procedures. In the presence of an increasing IgG, IgM, or IgA level, congenital hypogammaglobulinemia is unlikely. If the patient has been treated with γ-globulin to prevent severe or recurrent infection, then measurement of IgM and IgA assumes greater importance. Because commercial γ-globulin contains primarily IgG, the administration of γ-globulin will not affect serum levels of IgM and IgA. Increasing levels of these im-

munoglobulin classes indicate that the patient had transient hypogammaglobulinemia. Hypogammaglobulinemia may persist for as long as 2 years.

The cause of transient hypogammaglobulinemia is not known. In some cases, IgG anti-Gm antibodies have been demonstrated during the last trimester of pregnancy in women who have had infants with transient hypogammaglobulinemia. It is postulated that these antibodies cause suppression of endogenous immunoglobulin production in a manner similar to the suppression of normal red cell production in infants with passive transfer of antibody against Rh factors. Recent studies indicate that patients with transient hypogammaglobulinemia have normal numbers of B cells associated with a deficiency in number and function of helper T cells. Helper T cells become normal with time.

Occasionally, these infants become sufficiently symptomatic so that they must be treated just like those with X-linked hypogammaglobulinemia. γ-Globulin therapy may be required for as long as 18 months. Routine immunization should not be given during the period of transient hypogammaglobulinemia. Once a normal immune system has been established, the complete series of pediatric immunizations should be administered (see Chapter 37).

COMMON, VARIABLE, UNCLASSIFIABLE IMMUNODEFICIENCY (Acquired Hypogammaglobulinemia)

Major Immunologic Features

- Recurrent pyogenic infections with onset at any age.
- Increased incidence of autoimmune disease.
- Total immunoglobulins less than 300 mg/dL, with IgG less than 250 mg/dL.
- B cells usually normal in number.

General Considerations

Patients with acquired hypogammaglobulinemia present clinically like patients with X-linked hypogammaglobulinemia except that they usually do not become symptomatic until 15–35 years of age. In addition to increased susceptibility to pyogenic infections, they have a high incidence of autoimmune disease. These patients also differ from patients with congenital hypogammaglobulinemia in that they have a higher than normal incidence of abnormalities in T cell immunity, which in most instances shows progressive deterioration with time. Acquired hypogammaglobulinemia affects both males and females and may occur at any age.

Immunologic Pathogenesis

The cause of acquired hypogammaglobulinemia is unknown. Most patients have an intrinsic defect in B cells. Recent studies have demonstrated an inhibitory effect of peripheral blood lymphocytes from patients with acquired hypogammaglobulinemia on the immunoglobulin synthesis of cells from normal patients. A suggested increase in suppressor T cells has been postulated as a cause of this disorder. Other patients have diminished helper T cells. Some studies have shown a heterogeneity of arrested B cell development ranging from normal proliferative B cell responses and IgM-secreting cells to absent proliferative responses. Two enzymatic abnormalities have been described. In some patients there is a failure of heavy chain glycosylation of IgG. In others, a deficiency of 5′-nucleotidase has been described. The latter abnormality is most likely due to alterations in T:B cell ratios rather than a primary defect. An X-linked lymphoproliferative disorder associated with acquired hypogammaglobulinemia has been described following Epstein-Barr virus infection. Genetic studies of acquired hypogammaglobulinemia have demonstrated an autosomal recessive mode of inheritance in certain families in which abnormal lymphocyte metabolism has been shown to be inherited. In most instances, however, no clear-cut genetic transmission can be demonstrated. An increased incidence of other immunologic disorders, including autoimmune disease, has been observed in families of patients with acquired hypogammaglobulinemia. The presence of normal numbers of circulating peripheral blood B cells in most of these patients suggests that the disorder is a result of diminished synthesis or release of immunoglobulin rather than production of fewer immunoglobulin-synthesizing cells.

Clinical Features

A. Symptoms and Signs: In most instances, the initial presentation of acquired hypogammaglobulinemia consists of recurrent sinopulmonary infections. These may be chronic rather than acute and overwhelming, as in X-linked hypogammaglobulinemia. Infections may be caused by pneumococci and *H influenzae* as well as other pyogenic organisms. Chronic bacterial conjunctivitis may be an additional presenting complaint. Some patients develop severe malabsorption prior to the diagnosis of hypogammaglobulinemia. The malabsorption may be severe enough to cause protein loss, with the subsequent development of edema. Giardiasis, cholelithiasis, and achlorhydria are additional findings.

Autoimmune disease has been a presenting complaint in some patients with acquired hypogammaglobulinemia. A rheumatoid arthritis-like disorder, systemic lupus erythematosus (SLE), idiopathic thrombocytopenic purpura, dermatomyositis, hemolytic anemia, hypothyroidism, Graves' disease, and pernicious anemia have been reported in association with acquired hypogammaglobulinemia.

In contrast to patients with infantile X-linked hypogammaglobulinemia, patients with acquired hypogammaglobulinemia may have marked lymphadenopathy and splenomegaly. Intestinal lymphoid nodular hyperplasia has been described in association with malabsorption. Other abnormal physical findings relate to the presence of chronic lung disease or intesti-

nal malabsorption. Leukemia, lymphoma, and gastric carcinoma occur with increased frequency.

B. Laboratory Findings: Immunoglobulin measurements may show slightly higher IgG values than are reported in infantile X-linked hypogammaglobulinemia. Total immunoglobulins are usually less than 300 mg/dL, and IgG is usually less than 250 mg/dL. IgM and IgA may be absent or may be present in significant amounts. The Schick test is useful to demonstrate lack of normal antibody response, but it should be performed following booster immunization with diphtheria antigen. Isohemagglutinins are absent or present in low titers (< 1:10). The failure to produce antibody following specific immunization establishes the diagnosis in patients who have borderline immunoglobulin values. Live attenuated vaccines should not be utilized for immunization. Peripheral blood B lymphocytes are usually present in normal numbers in patients with acquired hypogammaglobulinemia—in contrast to patients with infantile X-linked hypogammaglobulinemia.

Although most patients with acquired hypogammaglobulinemia have intact cell-mediated immunity, a significant number demonstrate abnormalities as evidenced by absent delayed hypersensitivity skin test responses, depressed responses of isolated peripheral blood lymphocytes to PHA and allogeneic cells, and decreased numbers of T cell rosettes. NK cell activity is normal. A few patients have been found to have abnormal macrophage-T cell interaction. Patients should be followed sequentially, as the immunodeficiency appears to progressively involve cell-mediated immunity, resulting in additional immunologic deficiencies.

Biopsy of lymphoid tissue demonstrates a lack of plasma cells. Although some lymph node biopsies demonstrate lymphoid hyperplasia, there is a striking absence of cells in the B cell-dependent areas.

C. Other Studies: Other tests that may be abnormal in these patients relate to associated disorders. The chest x-ray usually shows evidence of chronic lung disease, and sinus films show chronic sinusitis. Pulmonary function studies are abnormal. Patients with malabsorption may have abnormal gastrointestinal tract biopsies, with blunting of the villi similar to that seen in celiac disease. Studies for malabsorption may indicate a lack of normal intestinal enzymes and an abnormal D-xylose absorption test. Occasionally, autoantibodies may be found in patients who have an associated autoimmune hemolytic anemia or SLE. In pernicious anemia, autoantibodies are not found, but biopsies of the stomach demonstrate marked lymphoid cell infiltration. Associated lymphoreticular malignancies and thymomas have been described.

Immunologic Diagnosis

The total immunoglobulin level is less than 300 mg/dL, with IgG less than 250 mg/dL. IgM and IgA may be absent or present in significant amounts. The antibody response following specific immunization is absent. Isohemagglutinins are depressed, and the Schick test is reactive. Circulating peripheral blood B cells are usually present in normal numbers but may be depressed.

Cell-mediated immunity may be intact or may be depressed, with negative delayed hypersensitivity skin tests, depressed responses of peripheral blood lymphocytes to PHA and allogeneic cells, and decreased numbers of circulating peripheral blood T cells. B cells may be normal or diminished in number in the peripheral blood. There is occasionally an increased number of null cells, ie, lymphocytes lacking surface markers for either T or B cells.

Differential Diagnosis

Occasionally the diagnosis of hypergammaglobulinemia in patients with infantile X-linked hypogammaglobulinemia may be delayed as long as 10 years. Because the treatment of infantile X-linked hypogammaglobulinemia and of acquired hypogammaglobulinemia is identical, this does not present a major clinical problem. Severe malabsorption associated with protein-losing enteropathy may be associated with hypogammaglobulinemia. These patients always have a simultaneous deficiency of serum albumin. A diagnosis of protein-losing enteropathy versus acquired hypogammaglobulinemia may be difficult under circumstances where protein-losing enteropathy is also associated with loss of lymphoid cells. In both groups of patients, depressed antibody responses and deficient cell-mediated immunity have been described. When the presenting feature of acquired hypogammaglobulinemia is an autoimmune disease, the diagnosis may be delayed, to the patient's detriment. In most instances, however, patients with autoimmune disease have normal to elevated immunoglobulin values. Patients with chronic lung disease should also be suspected of having cystic fibrosis, chronic allergy, α_1-antitrypsin deficiency, or immotile cilia syndrome.

Treatment

The treatment of acquired hypogammaglobulinemia is identical to that of infantile X-linked hypogammaglobulinemia. γ-Globulin in a dose of 20–40 mL/mo intramuscularly, fresh-frozen plasma (1–2 units/mo intravenously), and continuous antibiotics may be required in various combinations. Because of the large amounts of γ-globulin required, intramuscular injections may produce considerable discomfort. Intravenous γ-globulin is therefore quickly replacing intramuscular preparations. Special intravenous preparations, 100–400 mg/kg, can be given once each month. During acute illnesses, this amount can be given weekly or daily. Patients should be followed at regular intervals with chest x-rays and pulmonary function tests to determine adequacy of therapy. Pulmonary physical therapy is an essential part of treatment in patients with chronic lung disease.

Specific treatment of malabsorption problems may be required. Some patients respond to treatment with γ-globulin or with fresh-frozen plasma. Intravenous γ-globulin is now licensed for routine clinical use. Appropriate clinical application would be in patients

who fail to respond to intramuscular γ-globulin or who might benefit from large amounts of antibody. In others, the malabsorption may be associated with secondary enzymatic deficiencies that resemble celiac disease. These patients may respond to dietary restrictions. If the malabsorption is associated with *Giardia* infection, metronidazole therapy should be as for infantile X-linked hypogammaglobulinemia.

Caution should be exercised in the treatment of associated autoimmune disorders. The use of corticosteroids and immunosuppressive agents in a patient with immunodeficiency may result in markedly increased susceptibility to infection. Splenectomy has been used in the treatment of hypogammaglobulinemia and hemolytic anemia, but the mortality rate from overwhelming infection was high.

Complications & Prognosis

Patients with acquired hypogammaglobulinemia may survive to the seventh or eighth decade. Women with this disorder have had normal pregnancies and delivered normal infants (albeit hypogammaglobulinemic until 6 months of age). The major complication is chronic lung disease, which may develop despite adequate γ-globulin replacement therapy. An increased incidence of malignant disease has also been observed, including leukemia, lymphoma, and gastric carcinoma. Patients who develop acquired T cell deficiencies have increasing difficulty with infection characteristic of both T and B cell deficiencies. (See Table 20–1 parts A and B).

IMMUNODEFICIENCY WITH HYPER-IgM

This syndrome, characterized by an increased level of IgM (ranging from 150 to 1000 mg/dL) associated with a deficiency of IgG and IgA, is relatively rare and in most instances appears to be inherited in an X-linked manner. However, several cases have been reported of an acquired form that affects both sexes. The cause is not known. It has been postulated that in the normal individual there is a sequential development of immunoglobulins initiated by IgM production and subsequently resulting in the production of IgG and IgA (Chapter 5). Arrest in the development of immunoglobulin-producing cells after the formation of IgM-producing cells would be a possible cause.

Patients present with recurrent pyogenic infections, including otitis media, pneumonia, and septicemia. Some patients have recurrent neutropenia, hemolytic anemia, or aplastic anemia.

Laboratory evaluation reveals a marked increase in serum IgM, with absence of IgG and IgA. Isohemagglutinin titers may be elevated, and the patient may form antibodies following specific immunization. Detailed studies of cell-mediated immunity have not been performed, but some reports indicate that it is intact. Patients with this disorder may develop an infiltrating cancer of IgM-producing cells.

Treatment is similar to that of infantile X-linked hypogammaglobulinemia. Because few cases have been reported, it is difficult to determine the prognosis.

SELECTIVE IgA DEFICIENCY

Major Immunologic Features

- IgA less than 5 mg/dL with other immunoglobulins normal or increased.
- Cell-mediated immunity usually normal.
- Increased association with allergies, recurrent sinopulmonary infection, and autoimmune disease.

General Considerations

Selective IgA deficiency is the most common immunodeficiency disorder. The incidence in the normal population has been estimated to vary between 1:800 and 1:600. Considerable debate exists about whether individuals with selective IgA deficiency are "normal" or have significant associated diseases. Studies of individual patients and extensive studies of large numbers of patients suggest that absence of IgA predisposes to a variety of diseases. The diagnosis of selective IgA deficiency is established by finding an IgA level in serum of less than 5 mg/dL.

Immunologic Pathogenesis

The cause of selective IgA deficiency is not known. An arrest in development of B cells has been suggested based on the observation that B cells with both surface IgA and IgM or IgA and IgD are increased. An IgG2 subclass deficiency has been described in some patients and has been used to explain the varied clinical manifestations. The presence of normal numbers of circulating IgA B cells suggests that this disorder is associated with decreased synthesis or release of IgA rather than absence of IgA B lymphocytes. Utilizing the concept of sequential immunoglobulin production (IgM → IgG → IgA) discussed in Chapter 5, selective IgA deficiency could result from an arrest in the development of immunoglobulin-producing cells following the normal sequential development of IgM to IgG. The variety of diseases associated with selective IgA deficiency may be the result of enhanced or prolonged exposure to a spectrum of microbial agents and nonreplicating antigens as a consequence of deficient secretory IgA. The continuous assault by these agents on a compromised immune system could result in an increased incidence of infection, autoantibody, autoimmune disease, and cancer. Recently, an increased prevalence of HLA-A1, -B8, and -Dw3 has been found in patients with IgA deficiency and autoimmune disease.

Lymphocyte culture studies in IgA-deficient patients have demonstrated that IgA cells synthesize but fail to secrete IgA. Some individuals have suppressor T cells that selectively inhibit IgA production by normal lymphocytes.

Acquired IgA deficiency occurs frequently in patients treated with phenytoin or penicillamine and is

frequently associated with sinopulmonary tract infection. In at least some instances, the IgA level returns to normal when the drug therapy is stopped.

Clinical Features

A. Symptoms and Signs:

1. Recurrent sinopulmonary infection—The most frequent presenting symptoms are recurrent sinopulmonary viral or bacterial infections. Patients may occasionally present with recurrent or chronic right middle lobe pneumonia. Pulmonary hemosiderosis occurs with increased frequency and may be confused with chronic lung disease.

2. Allergy—In surveys of atopic populations the incidence of selective IgA deficiency is 1:400–1:200, as compared to an incidence of 1:800–1:600 in the normal population. Although the reasons for this association are not known, the absence of serum IgA may result in a significant reduction in the amount of antibody competing for antigens capable of combining with IgE. It is also possible that patients who lack IgA in their secretions may absorb intact proteins with an enhanced susceptibility to the formation of allergic responses. Allergic diseases in patients with selective IgA deficiency are often more difficult to control than the same allergies in other patients. It has also been the impression of several immunologists that these patients' symptoms are "triggered" by infection as well as by other environmental agents.

An increased incidence of antibody to bovine proteins, sometimes associated with circulating immune complexes, including bovine immunoglobulin, has been found in patients with selective IgA deficiency. This has been interpreted as providing additional evidence for abnormal gastrointestinal tract absorption. However, removal of milk from the diet has not been clearly associated with amelioration of symptoms.

A unique form of allergy exists in these patients. Certain patients with selective IgA deficiency develop high titers of antibody directed against IgA. Following the infusion of blood products, a few of these patients develop anaphylactic reactions. The incidence of antibodies directed against IgA in patients is much higher (30–40%) than the incidence of anaphylactoid transfusion reactions. Most patients who have anti-IgA antibodies have not had a history of γ-globulin or blood administration. Whether these antibodies are "autoantibodies" or antibodies resulting from sensitization is not certain. Possible sources of sensitization include breast milk feeding, passive transfer of maternal IgA, and cross-reaction with bovine immunoglobulin.

3. Gastrointestinal tract disease—An increased incidence of celiac disease has been noted. The disease may present at any time and is similar to celiac disease unassociated with IgA deficiency. Intestinal biopsies demonstrate an increase in IgM-producing cells. An anti-basement membrane antibody has been found with increased incidence.

Ulcerative colitis and regional enteritis have also been reported in association with selective IgA deficiency. Pernicious anemia has been reported in a significant number of patients. These patients have antibodies against both intrinsic factor and gastric parietal cells, which is not the case in hypogammaglobulinemia.

4. Autoimmune disease—A significant number of autoimmune disorders have been described in association with selective IgA deficiency. The disorders include SLE, rheumatoid arthritis, dermatomyositis, pernicious anemia, thyroiditis, Coombs-positive hemolytic anemia, Sjögren's syndrome, and chronic active hepatitis. Although the association of IgA deficiency and certain autoimmune disorders may be fortuitous, the increased incidence of IgA deficiency in SLE and rheumatoid arthritis is statistically significant (1:200–1:100).

The clinical presentation of patients with selective IgA deficiency and autoimmune disease does not appear to differ significantly from that of individuals with the identical disorder and normal or elevated levels of IgA. Because patients with selective IgA deficiency are capable of making normal amounts of antibody in the other immunoglobulin classes, they usually have characteristic autoantibodies associated with the specific autoimmune disease (antinuclear antibody, anti-DNA antibody, anti-parietal cell antibody, etc).

5. Selective IgA deficiency in apparently healthy adults—Because patients with selective IgA deficiency are capable of making normal amounts of antibody of the IgG and IgM classes, it is not surprising that many are entirely asymptomatic. However, long-term follow-up of some of these patients indicates that they may develop significant disease with time. There may be additional reasons why some patients remain asymptomatic. A small percentage of patients with selective IgA deficiency have normal amounts of secretory IgA and normal numbers of plasma cells containing IgA along the gastrointestinal tract. Some patients have increased amounts of low-molecular-weight (7S) IgM in their secretions. Finally, patients with selective IgA deficiency may have different exposures to pathogens and noxious agents in the environment.

6. Selective IgA deficiency and genetic factors—Both an autosomal recessive and an autosomal dominant mode of inheritance of IgA deficiency have been postulated. IgA deficiency appears with greater than normal frequency in families with other immunodeficiency disorders such as hypogammaglobulinemia. Partial deletion of the long or short arm of chromosome 18 (18q syndrome) or ring chromosome 18 has been described in selective IgA deficiency. However, many patients with abnormalities of chromosome 18 have normal levels of IgA in their serum. Selective IgA deficiency has been reported in one identical twin but not the other. In a study of familial IgA deficiency, an association with HLA-A2, B8, and Dw3 was described. Other studies have shown an increase in association with HLA-A1 and B8.

7. Selective IgA deficiency and cancer—Selective IgA deficiency has been reported in association with reticulum cell sarcoma, squamous cell carcinoma of the esophagus and lung, and thymoma. Several patients with IgA deficiency and cancer also had concomitant autoimmune disease and recurrent infection.

8. Selective IgA deficiency and drugs—Phenytoin and other anticonvulsants have been associated with selective IgA deficiency and in some cases with hypogammaglobulinemia. Many patients become symptomatic with recurrent sinopulmonary infections. Withdrawal of the drug does not always result in return to normal IgA values. The basic defect in these patients varies from normal to deficient IgA production in vitro. Deficient T cell-B cell interaction is found in some patients.

B. Laboratory Findings: Selective IgA deficiency has been defined as a serum level of IgA less than 5 mg/dL, with normal or increased values of IgG, IgM, IgD, and IgE. As there are a number of methods for measuring immunoglobulin levels, each laboratory should establish standards for detection of low values. Studies of B cell immunity indicate that these patients are capable of forming normal amounts of antibody following immunization. In most instances, absence of IgA in the serum is associated with absence of IgA in the secretions and with the presence of normal secretory component. Increased amounts of 7S IgM may be found in the serum and secretions. Abnormal κ/λ ratios are found. Evidence of abnormal antibody formation consists of an increased incidence of autoantibody, including antibodies directed against IgG, IgM, and IgA. The number of circulating peripheral blood B cells (including IgA-bearing B cells) is normal. Increased numbers of suppressor T cells have been reported in some patients.

Cell-mediated immunity is normal in most patients. Delayed hypersensitivity skin tests, the response of isolated peripheral blood lymphocytes to PHA and allogeneic cells, and the number of circulating T cells are normal. In a few patients, a depressed level of T cells, diminished production of T cell interferon, and decreased lymphocyte mitogenic responses have been found.

Other laboratory abnormalities are those typical of the associated diseases. Individuals who have chronic sinopulmonary infection may have abnormal x-rays and abnormal pulmonary function studies. Patients with IgA deficiency and celiac disease have abnormal gastrointestinal tract biopsies, abnormal D-xylose absorption, and an increased incidence of antibody directed against basement membrane. Patients with IgA deficiency and autoimmune disease have characteristic autoantibodies—eg, anti-DNA, antinuclear, antiparietal cell, a positive Coombs test. An increase in circulating immune complexes has been described.

Immunologic Diagnosis

Serum IgA and secretory IgA are absent in most patients. Some individuals have normal levels of secretory IgA. Other individuals have increased amounts of serum and secretory 7S IgM. A patient has been described with normal serum IgA levels and diminished secretory IgA, with absent secretory component. The antibody response to specific antigens is normal. Studies of cell-mediated immunity, including delayed hypersensitivity skin tests, response of peripheral blood lymphocytes to PHA and allogeneic cells, and numbers of circulating T cells, are usually normal.

Differential Diagnosis

Selective IgA deficiency must be distinguished from other, more severe immunodeficiency disorders associated with IgA deficiency. Forty percent of patients with ataxia-telangiectasia have selective IgA deficiency. These patients usually have cellular immunodeficiency as well. If IgA deficiency is found during the first years of life, a definitive diagnosis may not be possible because the complete ataxia-telangiectasia syndrome may not be present until the patient is 4–5 years old. Other immunodeficiency disorders that have been associated with selective IgA deficiency are chronic mucocutaneous candidiasis and cellular immunodeficiency with abnormal immunoglobulin synthesis (Nezelof's syndrome). A careful history should be obtained to rule out IgA deficiency secondary to drugs, eg, anticonvulsants or penicillamine.

Treatment

Patients with selective IgA deficiency *should not* be treated with γ-globulin therapy, since they are capable of forming normal amounts of antibody of other immunoglobulin classes and may recognize injected IgA as foreign. The use of γ-globulin in these patients enhances the risk of development of anti-IgA antibodies and subsequent anaphylactoid transfusion reactions. There is as yet no means by which the deficient IgA can be safely replaced. Patients with recurrent sinopulmonary infection should be treated aggressively with broad-spectrum antibiotics to avoid permanent pulmonary complications. Patients with SLE, rheumatoid arthritis, celiac disease, etc, respond to treatment (or not) in the same way as patients with the same diseases but no IgA deficiency. Transfusion reactions in patients with selective IgA deficiency may be minimized by several means. If the patient requires a blood transfusion, packed washed (3 times) red cells may be utilized. Although this does not completely eliminate the possibility of a transfusion reaction, it will decrease the risk. Alternatively, patients may be given blood from an IgA-deficient donor whose blood type matches the recipient's. Some immunologists have suggested freezing the patient's own plasma and red cells for future use.

Complications & Prognosis

Patients have survived to the sixth or seventh decade without severe disease. Most individuals, however, become symptomatic during the first decade of life. Recognition of the potential complications and prompt therapy for associated diseases will increase longevity and reduce the morbidity rate. Regular fol-

low-up examination is necessary for early detection of associated disorders and complications. A very few patients have developed normal IgA levels after years of IgA deficiency.

SELECTIVE IgM DEFICIENCY

Selective IgM deficiency is a rare disorder associated with the absence of IgM and normal levels of other immunoglobulin classes. IgM-bearing B cells are present in normal numbers. Some patients have decreased helper T cell activity. Some patients are capable of normal antibody responses in the other immunoglobulin classes following specific immunization, whereas others respond poorly. Cell-mediated immunity appears to be intact, but detailed studies have not been reported in a sufficient number of cases.

The cause of selective IgM deficiency is not known. The absence of IgM with presence of IgG and IgA appears to contradict the theory of sequential immunoglobulin development (see Chapter 5). The disorder has been described in both males and females.

Patients are susceptible to autoimmune disease and to overwhelming infection with polysaccharide-containing organisms (pneumococci, *H influenzae*). They may also have chronic dermatitis, diarrhea, and recurrent respiratory infections. Insufficient data are available to determine appropriate therapy. It would appear logical to manage these patients in a manner similar to the way an infant is managed following splenectomy, with immediate antibiotic (penicillin or ampicillin) treatment of all infections or with continuous antibiotic treatment. If patients are unable to form antibody to specific antigens, γ-globulin therapy should be given.

SELECTIVE DEFICIENCY OF IgG SUBCLASSES

Patients have been described with varying combinations of deficiency of the 4 IgG subclasses (IgG1, IgG2, IgG3, IgG4). Depending on the severity of the defect, the total serum IgG level may be normal or decreased. Serum levels of IgM and IgA are normal. Some patients respond with normal antibody production following immunization whereas others do not. All of the patients described suffered from repeated pyogenic sinopulmonary infections with pneumococci, *H influenzae*, and *Staphylococcus aureus*. Several patients reached the second decade of life before a diagnosis was established. All responded to treatment with intramuscular γ-globulin. The diagnosis is suggested by demonstrating an abnormal electrophoretic migration of IgG or by specific quantitation of IgG subclasses. The latter is not routinely available. IgG subclass deficiency may also be found in patients with IgA deficiency or ataxia-telangiectasia.

X-LINKED LYMPHOPROLIFERATIVE SYNDROME (Duncan's Syndrome)

The X-linked lymphoproliferative syndrome is associated with Epstein-Barr virus (EBV) infection. Following infection with EBV, several outcomes are possible: (1) fatal infectious mononucleosis, with severe liver disease and hepatitis accounting for most of the deaths; (2) fatal infectious mononucleosis with lymphoma; (3) infectious mononucleosis with immunodeficiency; and (4) lymphoma (possibly). Some patients may lack antibody to EBV in spite of documented infection. Others have an impaired antibody response especially to EBV nuclear antigen (EBNA). The immunodeficiency consists chiefly of acquired hypogammaglobulinemia. However, defects in the proliferative response of lymphocytes to mitogen and antigen, decreased NK cell activity, and abnormal T cell subset distribution have also been described. Some of these abnormalities are present only after severe EBV infection. Other findings include agranulocytosis and cardiovascular or central nervous system birth defects.

CELLULAR (T CELL) IMMUNODEFICIENCY DISORDERS

Immunodeficiency disorders associated with isolated defective T cell immunity are rare. In most patients, defective T cell immunity is associated with abnormalities of B cell immunity as well. This reflects the necessary collaboration between T and B cells before normal antibody formation can occur. Almost all patients with complete T cell deficiency have some impairment of antibody formation. Some patients with T cell deficiency have normal levels of immunoglobulin but fail to produce specific antibody following immunization. These patients are considered to have a qualitative defect in antibody production.

Patients with cellular immunodeficiency disorders are susceptible to a variety of microbial agents including viral, fungal, and protozoal infections. The infections may be acute or chronic.

Screening tests utilized to evaluate T cell immunity are listed in Table 20–3. The availability of newer techniques for the evaluation of T cell immunity has resulted in more precise diagnosis (Table 20–6) in many instances. (See also Chapter 18.)

CONGENITAL THYMIC APLASIA (DiGeorge Syndrome, Immunodeficiency With Hypoparathyroidism)

Major Immunologic Features

- **Congenital aplasia or hypoplasia of the thymus.**

- Lymphopenia reflects decreased numbers of T cells.
- Absent T cell function in peripheral blood.
- Variable antibody function.
- Successfully treated with thymus graft.

General Considerations

The DiGeorge syndrome is one of the few immunodeficiency disorders associated with symptoms immediately following birth. The complete syndrome consists of the following features: (1) abnormal facies consisting of low-set ears, "fish-shaped" mouth, hypertelorism, notched ear pinnae, micrognathia, and an antimongoloid slant of eyes; (2) hypoparathyroidism; (3) congenital heart disease; and (4) cellular immunodeficiency. Initial symptoms are related to associated abnormalities of the parathyroids and heart and may result in hypocalcemia and congestive heart failure. If the diagnosis of the DiGeorge syndrome is suspected because of these early clinical findings, then confirmation may be obtained by demonstrating defective T cell immunity. The importance of early diagnosis is related to the complete reconstitution of T cell immunity that can be achieved following a fetal thymus transplant.

Immunologic Pathogenesis

During the sixth to eighth weeks of intrauterine life, the thymus and parathyroid glands develop from epithelial evaginations of the third and fourth pharyngeal pouches. The thymus begins to migrate caudally during the 12th week of gestation. At the same time, the philtrum of the lip and the ear tubercle become differentiated along with other aortic arch structures. It is likely that the DiGeorge syndrome is the result of interference with normal embryologic development at approximately 12 weeks of gestation. In some patients, the thymus has not been absent but is in an abnormal location or is extremely small, though the histologic appearance is normal. It is possible that such patients represent "partial" DiGeorge syndrome in which hypertrophy of the thymus may take place with subsequent development of normal immunity. Following thymic transplantation, there is rapid T cell reconstitution, lack of GVH reaction, and lack of cellular chimerism, suggesting that patients lack a thymic humoral factor capable of expanding their own T cell immunity.

There is no inherited pattern associated with the DiGeorge syndrome, although a few cases have been described in the same family associated with chromosomal abnormalities (trisomy 20, monosomy 22). Recently, we have observed 4 DiGeorge patients with a history of maternal alcoholism. It is of interest that the fetal alcohol syndrome is associated with T cell deficiency.

Table 20–6. Evaluation of cell-mediated immunity.

Test	Comment
Total lymphocyte count	Normal at any age: >1200/μL.
Delayed hypersensitivity skin test	Used to evaluate specific immunity to antigens. Suggest *Candida*, mumps, PPD, and streptokinase-streptodornase. (4 units per 0.1 mL).
Lymphocyte response to mitogens (PHA), antigens, and allogeneic cells (mixed lymphocyte culture, MLC)	Used to evaluate T cell function. Results expressed as stimulated counts divided by resting counts equals stimulation index.
T cell rosettes (E rosettes)	Used to quantitate the number of circulating T cells. Normal: >60%.
Monoclonal antibody to T cells and T cell subsets	Determines total number of T cells as well as T cell subsets, eg, helper/suppressor.
Cytokine production (IL-1, IL-2, lymphotoxin, tumor necrosis factor, etc)	Used to detect specific cytokine production from subsets of mononuclear cells as an index of function.
Helper/suppressor T cell function	Determines helper/suppressor T cell numbers in vitro. Does not determine function.

Clinical Features

A. Symptoms and Signs: The most frequent presenting sign in patients with the DiGeorge syndrome is hypocalcemia in the first 24 hours of life that is resistant to standard therapy. Various types of congenital heart disease have been described, including interrupted aortic arch, septal defects, patent ductus arteriosus, and truncus arteriosus. Renal abnormalities may also be present. Some patients have the characteristic facial appearance described above. Patients who survive the immediate neonatal period may then develop recurrent or chronic infection with various viral, bacterial, fungal, or protozoal agents. Pneumonia, chronic infection of the mucous membranes with *Candida*, diarrhea, and failure to thrive may be present.

Spontaneous improvement of T cell immunity occasionally occurs. These patients are considered to have "partial" DiGeorge syndrome, but the reason for the spontaneous improvement in T cell immunity is not known. Patients have also been suspected of having the DiGeorge syndrome on the basis of hypocalcemia and congenital heart disease with or without the abnormal facies but have been found to have normal T cell immunity. Subsequently, these patients may develop severe T cell deficiency.

B. Laboratory Findings: Evaluation of T cell immunity can be performed immediately after birth in a patient suspected of having the DiGeorge syndrome. The lymphocyte count is usually low (< 1200/μL) but may be normal or elevated. In the absence of stress during the newborn period, a lateral view of the anterior mediastinum may reveal absence of the thymic shadow, indicating failure of normal development. Delayed hypersensitivity skin tests are of little value during early infancy, because sufficient time has not elapsed for sensitization to occur. T cells are markedly diminished in number, and the peripheral blood lymphocytes fail to respond to PHA and allogeneic cells.

Studies of antibody-mediated immunity are of little

value in early infancy, because immunoglobulins consist primarily of passively transferred maternal IgG. Although it is felt that some of these patients have a normal ability to produce specific antibody, the majority have some impairment of antibody formation. Sequential studies of both T and B cell immunity are necessary, since spontaneous remissions and spontaneous deterioration of immunity with time have been described.

A diagnosis of hypoparathyroidism is established by the demonstration of low serum calcium associated with an elevated serum phosphorus and an absence of parathyroid hormone. Congenital heart disease may be diagnosed immediately following birth and may be mild or severe. Other congenital abnormalities include esophageal atresia, bifid uvula, and urinary tract abnormalities.

Immunologic Diagnosis

T cell immunity is usually absent at birth as indicated by lymphocytopenia, depressed numbers of T cells, and no response of peripheral blood lymphocytes to PHA and allogeneic cells. Rarely, normal T cell immunity may develop with time, or previously normal T cell immunity may become deficient. In some patients, studies of T cell function are variable and range from depressed T cell numbers with normal function to a complete absence of T cell immunity.

Some patients have normal B cell immunity as indicated by normal levels of immunoglobulins and a normal antibody response following immunization. However, some patients with the DiGeorge syndrome have abnormal immunoglobulin values and fail to make specific antibody following immunization. Live attenuated viral vaccines should not be used for immunization. NK cell activity is normal.

Rarely, a patient may present with the immunologic features of severe combined immunodeficiency, ie, absent T and B cell immunity. The presence of hypocalcemia or congenital abnormalities of the third and fourth aortic arch establishes the diagnosis.

Differential Diagnosis

Many infants with severe congenital heart disease and subsequent congestive heart failure develop transient hypocalcemia. These infants should be suspected of having the DiGeorge syndrome. When the characteristic facial features are found, in addition to the hypocalcemia and congenital heart disease, an even stronger suspicion is present. Studies of T cell immunity will usually establish a diagnosis except in those infants with the DiGeorge syndrome who have developed effective T cell immunity with time. It is essential that all infants with congenital heart disease and hypocalcemia be followed until they are at least 1 year of age. The hypocalcemia associated with the DiGeorge syndrome is usually permanent, in contrast to that seen in congenital heart disease with congestive heart failure. Congenital hypoparathyroidism is usually not associated with congenital heart disease. However, both in this disorder and in the DiGeorge syndrome, low to absent levels of parathyroid hormone are present and the patients are resistant to the standard treatment of hypocalcemia. Low parathyroid hormone levels may also be found in transient hypocalcemia in infancy. Two patients with DiGeorge syndrome are known to have had spontaneous remissions of hypoparathyroidism.

Immunologic studies in a patient with the DiGeorge syndrome and in one with severe combined immunodeficiency disease may be identical in the newborn period. The presence of hypocalcemia, congenital heart disease, and an abnormal facies differentiates the DiGeorge syndrome from severe combined immunodeficiency disease.

Patients with the fetal alcohol syndrome may have similar facial and cardiac abnormalities as well as recurrent infections associated with decreased T cell immunity.

Treatment

A fetal thymus transplant should be given as soon as possible following diagnosis. This can result in permanent reconstitution of T cell immunity. The technique of thymus transplantation varies from local implantation in the rectus abdominis muscle to implantation of a thymus in a Millipore chamber. The thymus may also be minced and injected intraperitoneally. Because patients with the DiGeorge syndrome have been observed to develop a GVH reaction following administration of viable immunocompetent lymphocytes, fetal thymus glands older than 14 weeks' gestation should not be utilized. Thymocytes from glands younger than 14 weeks' gestation may lack cells capable of GVH reaction but can provide needed stem cells or thymic epithelial cells for further T cell development. Patients have been successfully treated with thymosin and thymus epithelial transplants.

The hypocalcemia is rarely controlled by calcium supplementation alone. Calcium should be administered orally in conjunction with vitamin D or parathyroid hormone.

Congenital heart disease frequently results in congestive heart failure and may require immediate surgical correction. If surgery is performed prior to the availability of a fetal thymus transplant, any blood given should be irradiated with 3000 R to prevent a GVH reaction.

Complications & Prognosis

Prolonged survivals have been reported following successful thymus transplantation or spontaneous remission of immunodeficiency. Sudden death may occur in untreated patients or in patients initially found to have normal T cell immunity. Congenital heart disease may be severe, and the infant may not survive surgical correction. Death from GVH disease following blood transfusions has been observed in patients in whom a diagnosis of the DiGeorge syndrome was not suspected.

CHRONIC MUCOCUTANEOUS CANDIDIASIS (With & Without Endocrinopathy)

Major Immunologic Features

- Onset may be either with chronic candidal infection of the skin and mucous membranes or with endocrinopathy.
- Delayed hypersensitivity skin tests to *Candida* antigen are negative in spite of chronic candidal infection.

General Considerations (See also Chapter 29.)

Chronic mucocutaneous candidiasis affects both males and females. A familial occurrence has been reported, suggesting an autosomal recessive inheritance. The disorder is associated with a selective defect in T cell immunity, resulting in susceptibility to chronic candidal infection. B cell immunity is intact, resulting in a normal antibody response to *Candida* and, in some patients, the development of autoantibodies associated with idiopathic endocrinopathies. The disorder may appear as early as 1 year of age or may be delayed until the second decade.

Various theories have been proposed to explain the association of chronic candidal infection and the development of endocrinopathy. Initially it was felt that hypoparathyroidism predisposed to candidal infection. Subsequently it was found that many patients developed severe candidal infection without evidence of hypoparathyroidism. A basic autoimmune disorder has been postulated with the suggestion that the thymus also functions as an endocrine organ and that the thymus and other endocrine glands are involved in an autoimmune destructive process.

Clinical Features

A. Symptoms and Signs: The initial presentation of chronic mucocutaneous candidiasis may be either chronic candidal infection or the appearance of an idiopathic endocrinopathy. If candidal infection appears first, several years to several decades may elapse before endocrinopathy occurs. Other patients may present with the endocrinopathy first and subsequently develop candidal infection. Candidal infection may involve the mucous membranes, skin, nails, and, in older patients, the vagina. In severe forms, infection of the skin occurs in a "stocking-glove" distribution and is associated with the formation of granulomatous lesions. Patients are usually not susceptible to systemic candidal infection. Rarely, they may develop infection with other fungal agents.

Other symptoms are related to the development of a specific endocrinopathy. Hypoparathyroidism is the most common and is associated with hypocalcemia and tetany. Addison's disease is the next most common. A variety of other endocrinopathies have been reported, including hypothyroidism, diabetes mellitus, and pernicious anemia. Occasionally, there is a history of acute or chronic hepatitis preceding the onset of endocrinopathy. Additional disorders include pulmonary fibrosis, ovarian failure, ACTH deficiency, and keratoconjunctivitis.

B. Laboratory Findings: Studies of T cell immunity reveal a specific though variable defect in T cell immunity. Patients usually have a normal total lymphocyte count; isolated peripheral blood lymphocytes respond to PHA and to allogeneic cells and to antigens other than *Candida*. The least severe T cell defect is an absent delayed hypersensitivity skin test response to *Candida* antigen in the presence of documented chronic candidiasis. Other patients may have additional defects, including the inability to form migration inhibitory factor (MIF) in response to *Candida* antigens or inability of lymphocytes to be activated by *Candida* antigens and decreased suppressor T cell activity. B cell immunity is intact, as demonstrated by the presence of normal or elevated levels of immunoglobulins, increased amounts of antibody directed against *Candida*, and autoantibody formation. Occasionally, selective absence of IgA or elevated levels of immunoglobulins may be observed. Plasma inhibitors of T cell function and increased numbers of suppressor T cells have been reported in some cases. Isolated cases have been reported with chemotaxis or macrophage abnormalities.

Other laboratory abnormalities are related to the presence of endocrinopathies. Hypoparathyroidism is associated with decreased serum calcium, elevated serum phosphorus, and low or absent parathyroid hormone. Increased skin pigmentation may herald the onset of Addison's disease prior to disturbances in serum electrolytes. An ACTH stimulation test is useful to document the presence of Addison's disease. Other abnormalities of endocrine function include hypothyroidism, abnormal vitamin B_{12} absorption, and diabetes mellitus. Abnormal liver function studies may indicate chronic hepatitis. Occasionally, iron deficiency is present which, when treated, results in improvement in the candidal infection. Autoantibodies associated with specific endocrinopathy are usually present before and during the development of endocrine dysfunction. They may be absent when complete endocrine deficiency is present. Patients should be evaluated on a yearly basis for endocrine function, because the endocrinopathies are progressive.

Immunologic Diagnosis

Major aspects of T cell immunity are normal, as indicated by a normal response of peripheral blood lymphocytes to PHA and allogeneic cells. Activation of lymphocytes and MIF production in response to antigens other than *Candida* is normal. T cells are present in normal numbers. In some patients, only the delayed hypersensitivity skin test response to *Candida* antigens is absent. Other patients have an absence of MIF production or an absence of lymphocyte activation by *Candida* antigens. Plasma inhibitors of cellular immunity may also occur. B cell immunity is intact, with normal production of antibody to *Candida*.

Differential Diagnosis

Children with chronic candidal infection of the mucous membranes may have a variety of immunodeficiency disorders. Detailed studies of B and T cell immunity differentiate between chronic mucocutaneous candidiasis, in which there is a selective deficiency of T cell immunity to *Candida* antigens, and other disorders where T cell immunity may be completely deficient. Patients with the DiGeorge syndrome (thymic aplasia and hypoparathyroidism) present early in infancy, whereas chronic mucocutaneous candidiasis with hypoparathyroidism is a disorder of later onset and progressive nature. Patients with late-onset idiopathic endocrinopathies should be considered to have chronic mucocutaneous candidiasis even though candidal infection is not present at the time of diagnosis. These patients may develop chronic candidal infection as late as 10–15 years after the onset of endocrinopathy.

Treatment

There is no treatment to prevent the development of idiopathic endocrinopathy. The physician must be alert to the gradual development of endocrine dysfunction—particularly Addison's disease, which is the major cause of death. Chronic skin and mucous membrane candidal infection is difficult to treat. Topical treatment with a variety of antifungal agents has been attempted but has usually been unsuccessful. Local miconazole therapy has provided control in some patients in recent trials. This drug has also been used intravenously on an experimental basis with some success. Courses of intravenous amphotericin B have resulted in improvement in a significant number of patients, but this form of treatment is limited by the renal toxicity of the drug. Oral clotrimazole is occasionally of benefit. Oral ketoconazole, an antifungal agent, offers additional promise. The combination of transfer factor therapy subcutaneously and amphotericin B intravenously has been successful in approximately 50% of patients. Transfer factor is obtained from normal *Candida* skin test-positive donors and administered before or during—or both before and during—amphotericin therapy. Two patients refractory to all other forms of therapy were successfully treated with fetal thymus transplants.

Complications & Prognosis

Patients may survive to the second or third decade but usually experience extensive morbidity. Individuals with severe candidal infection of the mucous membranes and skin develop serious psychologic difficulties. Systemic infection with *Candida* usually does not occur. Rarely, patients may develop systemic infection with other fungal agents. Hypoparathyroidism is difficult to manage, and complications are frequent. Addison's disease is the major cause of death and may develop suddenly without previous symptoms.

COMBINED ANTIBODY-MEDIATED (B CELL) & CELL-MEDIATED (T CELL) IMMUNODEFICIENCY DISEASES

Combined immunodeficiency diseases are due to various causes and are of variable severity. Defective T cell and B cell immunity may be complete, as in severe combined immunodeficiency disease, or partial, as in ataxia-telangiectasia. The association of distinct clinical features in ataxia-telangiectasia serves to further differentiate the disorder from severe combined immunodeficiency disease and also suggests that the causes of these disorders are not the same. Enzymatic deficiencies in the purine pathway have been described in association with combined immunodeficiency disease. This discovery has provided additional evidence for a diverse origin of combined immunodeficiency diseases.

Studies of both T and B cell immunity are necessary to completely evaluate patients with combined immunodeficiency disorders (Tables 20–3, 20–5, and 20–6). In addition, analysis of red and white cell enzymes (adenosine deaminase and nucleoside phosphorylase) may provide additional information for appropriate classification.

The onset of symptoms in patients with combined immunodeficiency diseases is usually early in infancy. They are susceptible to a very wide spectrum of microbial agents. Immunotherapy is frequently difficult and often not available.

SEVERE COMBINED IMMUNODEFICIENCY DISEASE

Major Immunologic Features

- Onset of symptoms by 6 months of age, with recurrent viral, bacterial, fungal, and protozoal infection.
- Occurs in X-linked, autosomal, and sporadic forms.
- Absence of both T and B cell immunity.

General Considerations

The immunologic deficiency includes absence of T and B cell immunity, resulting in early susceptibility to infection with virtually any type of microbial agent. Patients rarely survive beyond 1 year of age before succumbing to one or more opportunistic infections. The disease is inherited in 2 forms: an X-linked recessive form (X-linked lymphopenic agammaglobulinemia) and an autosomal recessive form (Swiss-type lymphopenic agammaglobulinemia). The exact incidence of this disorder is not known, and many patients die before the diagnosis is made. Because the immune system of patients with this disorder may be made completely normal by bone marrow transplantation,

early diagnosis is urgent to prevent irreversible complications.

Immunologic Pathogenesis

The basic defect is not known, but it has been postulated that severe combined immunodeficiency disease is a result of failure of differentiation of stem cells into T and B cells. The successful use of histocompatible bone marrow transplantation has provided support for the concept of a basic stem cell defect. However, some authors argue that the defect may reside in failure of the thymus and bursa equivalent to develop normally. Normal stem cells would not be processed into T and B cells under these circumstances. Others argue for an intrinsic defect within the thymus based on in vitro maturation of patients' T cells when cultured in the presence of thymus epithelium or the presence of immature T cells defined by monoclonal antibody.

Clinical Features

A. Symptoms and Signs: Patients with severe combined immunodeficiency disease usually succumb to overwhelming infection within the first year of life. Early findings include failure to thrive, chronic diarrhea, persistent thrush (oral candidiasis), pneumonia, chronic otitis media, and sepsis. The microbial agents that result in acute and chronic infection include viruses, bacteria, fungi, and protozoa. Infants with this disease are particularly susceptible to *Candida*, cytomegalovirus, and *Pneumocystis carinii* infection. When smallpox immunization was administered routinely, many of these infants developed progressive vaccinia. Death from progressive poliomyelitis following attenuated viral immunization has been documented. During the first several months of life, patients may be partially protected from bacterial infections by the passive transfer of maternal IgG. They subsequently develop susceptibility to a wide variety of gram-positive and gram-negative organisms.

As these patients entirely lack T cell immunity, they are susceptible to GVH reactions that may develop following maternal infusion of cells during gestation or delivery, infusion of viable cells in the form of blood transfusions, or attempts at immunotherapy. (See section on GVH disease, below.) The presence of an acute or chronic GVH reaction may complicate the diagnosis of severe combined immunodeficiency disease. Some of these patients have been misdiagnosed as having an acute viral illness, histiocytosis X, or other chronic disorders.

Physical findings relate to the degree and type of infections present. Pneumonia, otitis media, thrush, dehydration, skin infections, and developmental retardation may be present. Lymphoid tissue and hepatosplenomegaly are absent unless the disease is complicated by a GVH reaction.

B. Laboratory Findings: All tests of T cell immunity are abnormal. The thymus shadow is absent, lymphopenia is usually present, T cells are markedly depressed, and the response of isolated peripheral blood lymphocytes to PHA and allogeneic cells is absent. Rarely, some patients have a proliferative response to allogeneic cells. T cell subset analysis varies considerably. T cell-specific antigens are absent in some patients, while others show developmental arrests at the prothymocyte level. Delayed hypersensitivity skin tests are not useful for diagnosis, because in most cases insufficient time has elapsed for cellular sensitization to occur. During the first 5–6 months of life, a diagnosis of severe combined immunodeficiency disease may be difficult to establish because of the presence of maternal IgG. However, most normal infants who have had repeated infection will develop significant amounts of serum IgM or IgA (or both). If the diagnosis is doubtful, it may be necessary to specifically immunize a patient and determine specific antibody responses. Patients suspected of having immunodeficiency diseases should never be immunized with attenuated live virus vaccines. In the majority of patients with severe combined immunodeficiency, B cells are absent or markedly reduced from birth. A subgroup of patients exists in which B cells may be elevated. NK cell activity may be normal or deficient. Adenosine deaminase activity is normal.

Biopsy of lymphoid tissue is rarely necessary to establish a diagnosis. If biopsies are obtained, they should be performed cautiously, because secondary infection is frequent. Biopsy of lymph nodes (if they can be found) demonstrates lymph nodes severely depleted of lymphocytes, without corticomedullary differentiation and without follicle formation. Biopsy of the intestinal tract shows a complete absence of plasma cells.

Patients who have pulmonary infiltrates which do not respond to antibiotic therapy or which are associated with rapid respiration and a low P should be suspected of having *P carinii* infection. Because this disorder can be treated, it is important to establish an early diagnosis. Some debate exists about whether the diagnosis is best made by means of concentrated sputum examination, bronchoscopy, needle biopsy, or open lung biopsy. In most instances, open lung biopsy provides the most complete information. Cytomegalovirus infection should be considered in all patients. Cultures of blood; mucous membranes, and stool for predominant bacterial organisms may be important in determining subsequent treatment. Individuals who have been inadvertently immunized with live poliovaccine should have stools cultured for poliovirus.

Patients frequently have anemia and lymphopenia. Complications such as chronic systemic infection and GVH reaction may result in multiple abnormalities including elevation of liver enzymes, jaundice, chronic diarrhea with subsequent electrolyte abnormalities and dehydration, pulmonary infiltrates, cardiac irregularities, and abnormal cerebrospinal fluid analysis.

Immunologic Diagnosis

Severe combined immunodeficiency disease is associated with complete absence of T and B cell immunity. Evaluation of T cell immunity reveals lymphope-

nia, absence of thymus shadow, depressed T cells, absence of peripheral blood lymphocyte responses to PHA, allogeneic cells, and antigens, and absence of response to delayed hypersensitivity skin tests. Evaluation of B cell immunity reveals hypogammaglobulinemia, absence of antibody response following immunization, and depressed or absent numbers of circulating B cells (rarely, elevated B cells).

Differential Diagnosis

Severe combined immunodeficiency disease must be differentiated from other immunodeficiency disorders with defects in T and B cell immunity. The early onset of symptoms and the complete absence of both T and B cell immunity found in severe combined immunodeficiency disease usually result in a specific diagnosis. Combined immunodeficiency associated with absence of an enzyme in the purine pathway (adenosine deaminase) has been described. This disorder is usually less severe initially clinically and immunologically. The presence of a GVH reaction in severe combined immunodeficiency may complicate the diagnosis. If chronic dermatitis is present in association with hepatosplenomegaly and histiocytic infiltration, a mistaken diagnosis of Letterer-Siwe syndrome may be made. Some patients may present with chronic diarrhea and pigmentary skin changes resulting in an erroneous diagnosis of acrodermatitis enteropathica. (See discussion of GVH disease, below.)

Treatment

Aggressive diagnostic measures are necessary to establish the cause of chronic infection before treatment can be instituted. Open lung biopsy should be performed if *P carinii* infection is suspected. The treatment of choice for *Pneumocystis* consists of pentamidine and trimethoprim-sulfamethoxazole given simultaneously. Specific antibiotic treatment is necessary for suspected bacterial infection. Superficial candidal infection is treated with topical antifungal agents, but systemic infection requires intravenous amphotericin B therapy or ketoconazole.

Complications must be avoided. Live attenuated viral immunization should not be performed. Blood products containing potentially viable lymphocytes should be irradiated with 3000–6000 R prior to administration. (See section on GVH disease, below.)

During the initial period of evaluation, γ-globulin may be administered in doses of 0.2–0.4 mL/kg given once each month or as frequently as once each week. Severely ill patients appear to improve with the use of intravenous γ-globulin in doses of 100–400 mg/kg given every 1–4 weeks. Definitive treatment consists of transplantation of histocompatible bone marrow. Because of the inheritance of the histocompatibility antigens, the usual donor for a bone marrow transplant is a histocompatible sibling. The bone marrow must be matched by both the HLA and MLC tests. (See Chapters 6, 18, and 23.) Despite careful matching, a GVH reaction may develop. Several techniques have been utilized in performing bone marrow transplantation, including intraperitoneal injection and intravenous infusion of filtered bone marrow. The dose of bone marrow cells administered has varied, but as few as 1000 nucleated cells per kilogram have resulted in successful immunologic reconstitution. Transplantation of unmatched marrow has previously resulted in a fatal GVH reaction. Recently, some investigators have utilized HLA-nonidentical, MLC-identical bone marrow from nonsibling donors, or MLC-nonidentical marrow prepared by lectin separation of cells or bone marrow treated with monoclonal antibody to reduce GVH potential. Preliminary results with these techniques are promising.

In the absence of a histocompatible bone marrow donor, other forms of therapy have been utilized. Long-term survivors of fetal liver transplants (< 8 weeks' gestation) and of fetal thymus transplantation (< 14 weeks' gestation) have been observed. In both of these techniques, the use of older fetal liver or thymus will result in a fatal GVH reaction, probably because of the presence of mature immunocompetent cells. Thymus epithelial transplants and combined fetal liver and thymus transplants have also been used.

Complications & Prognosis

Patients with severe combined immunodeficiency disease are unusually susceptible to infection with many microbial agents and will succumb prior to 1 year of age if untreated. If the diagnosis is not made immediately, the patient may receive live attenuated viral immunization and succumb to progressive vaccinia or poliomyelitis. In other instances, patients may receive unirradiated blood products and die from complications of GVH disease. Following successful bone marrow transplantation, 10-year survivals with maintenance of normal T and B cell function have been recorded. Patients have survived as long as 1 year following fetal liver transplantation and as long as 6 years following fetal thymus transplantation. The reconstitution of immunity in patients receiving fetal organ transplantation has not been complete.

CELLULAR IMMUNODEFICIENCY WITH ABNORMAL IMMUNOGLOBULIN SYNTHESIS (Nezelof's Syndrome)

Major Immunologic Features

- Susceptibility to viral, bacterial, fungal, and protozoal infection
- Absent to depressed T cell immunity.
- Various degrees of B cell immunodeficiency associated with various combinations of increased, normal, or decreased immunoglobulin levels.

General Considerations

The disorders included in this classification are diverse and probably do not all have the same cause. Consistent features include marked deficiency of T

cell immunity and varying degrees of deficiency of B cell immunity. Disorders with specific clinical symptomatology such as ataxia-telangiectasia and Wiskott-Aldrich syndrome or associated with enzyme deficiency such as adenosine deaminase are excluded. Part of the difficulty in defining disorders in this category relates to recent developments in the diagnosis of T cell immunity that were not available when some of these cases were first reported. Most of the cases included in this category are sporadic and do not have a definite inherited pattern.

Immunologic Pathogenesis

The cause is not known. There appears to be no specific genetic pattern, and the disease is sporadic in distribution and occurs in both males and females. The presence of moderate to severe deficiencies of T cell immunity with varying degrees of B cell immunodeficiency suggests that the primary defect is within the thymus. It is possible that this disorder is the result of thymic hypoplasia with deficient interaction of T and B cells and subsequent abnormal antibody formation. Patients with epidemiologic and laboratory features of acquired immunodeficiency syndrome (AIDS) should be excluded (see pp 347–353).

Clinical Features

A. Symptoms and Signs: Patients are susceptible to recurrent fungal, protozoal, viral, and bacterial illnesses. The spectrum of infection is similar to that found in patients with congenital hypogammaglobulinemia and other forms of combined immunodeficiency. Patients frequently have marked lymphadenopathy and hepatosplenomegaly, in contrast to patients with congenital hypogammaglobulinemia and severe combined immunodeficiency disease.

B. Laboratory Findings: Studies of T cell immunity are abnormal, but the degree of deficiency may vary. Lymphopenia may be present, but occasionally a normal lymphocyte count is obtained. T cells are moderately to markedly decreased. The lymphocyte response to PHA and specific antigens may be absent or slightly depressed, and the lymphocyte response to allogeneic cells may vary from nil to normal. B cell immunity is abnormal. The 5 immunoglobulin classes may be present in varying combinations of increased, normal, or decreased amounts. Total circulating B cells are usually present in normal numbers, although the distribution among various types of surface immunoglobulin-bearing B cells may vary. Despite the presence of normal or elevated levels of immunoglobulin, there is no antibody response following specific immunization. Antibody to specific substances may be found, however, indicating that at one time some of these patients may have been able to form antibody. Isohemagglutinins may be absent or normal, and the Schick test may be reactive or nonreactive.

Biopsy of lymphoid tissue in these patients may reveal the presence of plasma cells. The lymph nodes may be large and may contain numerous histiocytes and macrophages with granuloma formation.

Immunologic Diagnosis

The principal immunologic features in this group of disorders consist of moderate to marked reductions in numbers of total lymphocytes and T cells and a diminished response of peripheral blood lymphocytes to PHA, allogeneic cells, and specific antigens. There is usually no response to delayed hypersensitivity skin tests. Variable degrees of B cell deficiency are present, consisting of varying combinations of elevated, normal, or low levels of specific immunoglobulin classes. The antibody response to specific antigens is usually absent. Some evidence of prior antibody formation may be found, eg, nonreactive Schick test, isohemagglutinins. The number of total circulating B cells is usually normal.

Differential Diagnosis

Because there is a lack of uniformity in the clinical and laboratory presentation of these patients, it is necessary to rule out other disorders with definite clinical or laboratory associations. The clinical features of ataxia-telangiectasia are usually present by 3–4 years of age, and an elevated alpha-fetoprotein is usually present by 1 year of age. Patients with Wiskott-Aldrich syndrome have thrombocytopenia from birth and can be excluded on this basis. Patients with severe combined immunodeficiency disease have complete absence of T and B cell immunity. Immunodeficiency disorders associated with enzyme deficiencies may have a similar presentation and are excluded on the basis of enzyme analysis of red or white blood cells. Patients with short-limbed dwarfism are excluded on the basis of characteristic clinical and radiologic features. Patients with cellular immunodeficiency and abnormal immunoglobulin synthesis do not develop endocrine abnormalities and can therefore be distinguished from patients with the DiGeorge syndrome and chronic mucocutaneous candidiasis who are capable of normal antibody synthesis. It may be difficult to distinguish a patient with AIDS from one with this disorder.

Treatment

Aggressive treatment of infection is necessary. Patients failing to show an antibody response after immunization (even if immunoglobulin levels are normal) should receive monthly γ-globulin administration. (See treatment of hypogammaglobulinemia, above.) Continuous broad-sprectrum antibiotic coverage may be useful. Postural drainage is important to prevent chronic lung disease.

Although histocompatible bone marrow transplantation would appear to be curative in these patients, few successful cases have been reported. This appears to be due to lack of histocompatible donors rather than the complications of transplantation. Transfer factor therapy has been utilized with questionable success. Thymus transplantation and the use of several thymus factors have been reported to provide reconstitution of T cell immunity and partial reconstitution of B cell immunity.

Patients should not be immunized with attenuated

viral vaccines. All blood products should be irradiated with 3000 R.

Complications & Prognosis

Patients do not develop the severe complications observed in severe combined immunodeficiency disease. GVH reaction has not been reported. Some of these patients, however, may develop progressive encephalitis following live attenuated viral immunization. Chronic lung disease, chronic fungal infection and later development of cancer are long-term complications. Survival until age 18 has been recorded.

IMMUNODEFICIENCY WITH ATAXIA-TELANGIECTASIA

Major Immunologic Features

- Clinical onset by 2 years of age.
- Complete syndrome consists of ataxia, telangiectasia, and recurrent sinopulmonary infection.
- Selective IgA deficiency present in 40% of patients.

General Considerations

Ataxia-telangiectasia is inherited in an autosomal manner. It is associated with characteristic features, including ataxia, telangiectasis, recurrent sinopulmonary infection, and abnormalities in both T and B cell immunity. The disorder was first considered to be primarily a neurologic disease and is now known to involve the neurologic, vascular, endocrine, and immune systems.

Immunologic Pathogenesis

There is no unifying theory that explains the multisystem abnormalities present in ataxia-telangiectasia. A defect in the development of mesoderm has been postulated but not confirmed. Other abnormalities described that may account for some of the multisystem disorders are abnormal collagen deficient in hydroxylysine, elevated alpha-fetoprotein indicative of a defect in organ maturation, enhanced susceptibility of cells to radiation damage, and defective DNA repair. Clones of lymphocytes with structural rearrangement of the long arm (q) of chromosome 14 have been consistently found. This chromosome marker may be found in malignant cell lines isolated from patients. The disorder is progressive, with both the neurologic abnormalities and the immunologic deficiency becoming more severe with time.

Clinical Features

A. Symptoms and Signs: Ataxia may have its onset at 9 months to 1 year of age or may be delayed as long as 4–6 years. Telangiectasia is usually present by 2 years of age but has been delayed until 8–9 years of age. As patients become older, additional neurologic symptoms develop, consisting of choreoathetoid movements, disconjugate gaze, and extrapyramidal and posterior column signs. Telangiectasia may develop first in the bulbar conjunctiva and subsequently appear on the bridge of the nose, the ears, or in the antecubital fossae. Recurrent sinopulmonary infections may begin early in life, or patients may remain relatively symptom-free for 10 years or more. Susceptibility to infection includes both viral and bacterial infections. Secondary sexual characteristics rarely develop in patients at puberty, and most patients appear to develop mental retardation with time.

B. Laboratory Findings: Varying degrees of abnormalities in T and B cell immunity have been described. Lymphopenia may be present, T cells may be normal or decreased, and the response of lymphocytes to PHA and allogeneic cells may be normal or decreased. There may be no response to delayed hypersensitivity skin tests. Serum IgA is absent in approximately 40% of patients. IgG4 and IgA2 subclass deficiency is present in some patients. In still other patients, IgE may be absent. Antibody responses to specific antigens may be depressed. The number of circulating B cells is usually normal. NK cell activity is normal.

Other laboratory abnormalities relate to associated findings. Abnormalities have been shown on pneumoencephalography, electromyography, and electroencephalography. Endocrine studies have shown decreased 17-ketosteroids and increased FSH excretion. An insulin-resistant form of diabetes has been described. Liver function tests are abnormal. An increased incidence of autoantibodies has been found. Cytotoxic antibodies to brain and thymus have been found. Increased levels of alpha-fetoprotein have been described in all patients tested and may be specific for this disease. Unfortunately, alpha-fetoprotein levels are high in normal infants until 1 year of age. Many patients have elevated titers to EBV antigens.

Immunologic Diagnosis

Selective IgA deficiency is found in 40% of patients. IgA2, IgG2, and IgG4 subclass deficiency has also been described. IgE deficiency and variable deficiencies of other immunoglobulins may also be found. The antibody response to specific antigens may be depressed. Variable degrees of T cell deficiency are observed which usually become more severe with advancing age.

Differential Diagnosis

If the onset of recurrent infection occurs before the development of ataxia or telangiectasia, it may be difficult to differentiate this disorder from cellular immunodeficiency with abnormal immunoglobulin synthesis. If a patient has a gradual onset of cerebellar ataxia unassociated with telangiectasis and immunologic abnormalities, one may have to wait years before a diagnosis can be established with certainty. Usually, by age 4, the characteristic recurrent sinopulmonary infections, immunologic abnormalities, ataxia, and telangiectasia are present concomitantly. Because selective IgA deficiency is the most common im-

munodeficiency disorder detected and many patients with selective IgA deficiency have no associated symptomatology, one may have to wait several years before a diagnosis of ataxia-telangiectasia can be excluded. Alpha-fetoprotein levels are normal in patients with IgA deficiency.

Treatment

Early treatment of recurrent sinopulmonary infections is essential to avoid permanent complications. Some patients may benefit from continuous broad-spectrum antibiotic therapy. In patients who develop chronic lung disease, physical therapy with postural drainage is of benefit. Transfer factor has been utilized in some patients without apparent benefit. Successful bone marrow transplantation has not been performed, but this is probably related to the lack of histocompatible bone marrow donors. Fetal thymus transplantation has provided some benefit in a limited number of patients. Thymic factor therapy has been used to treat a limited number of patients. The use of intravenous γ-globulin in a patient unable to form antibody may result in a reduced number of infections (see treatment of hypogammaglobulinemia, above).

Attenuated viral vaccines should not be given. All blood products should be irradiated with 3000 R prior to administration.

Complications & Prognosis

Patients who survive for long periods of time develop progressive deterioration of neurologic and immunologic functions. The oldest patients have reached the fifth decade of life. The chief causes of death are overwhelming infection and the development of lymphoreticular or epithelial cell cancer. Leukemias with associated abnormalities of chromosome 14 have been reported. As these patients reach the second decade, morbidity becomes severe, with chronic lung disease, mental retardation, and physical debility the principal problems. Heterozygote carriers as well as family members have an increased incidence of cancer.

IMMUNODEFICIENCY WITH THROMBOCYTOPENIA, ECZEMA, & RECURRENT INFECTION (Wiskott-Aldrich Syndrome)

Major Immunologic Features

- Complete syndrome consists of eczema, recurrent pyogenic infection, and thrombocytopenia.
- Can be diagnosed at birth by demonstration of thrombocytopenia in a male infant with a positive family history.
- Serum IgM usually low with elevated serum IgA and IgE.

General Considerations

Patients may become symptomatic early in life, with bleeding secondary to thrombocytopenia. Subsequently they develop recurrent bacterial infection in the form of otitis media, pneumonia, and meningitis. Eczema usually appears by 1 year of age. The disease appears to be progressive, with increasing susceptibility to infection. It is inherited in an X-linked manner.

Immunologic Pathogenesis

Two of the earliest abnormalities found in patients are thrombocytopenia and hypercatabolism of immunoglobulin. A hypothesis linking thrombocytopenia, eczema, and recurrent infection is not available. It has been suggested that the α granules of platelets and macrophages of patients and carriers are abnormal. Another suggestion is that the inability of patients to respond to polysaccharide antigens results in immunologic attrition. This does not explain the thrombocytopenia or eczema. There are reports of absence of a 115,000-MW surface glycoprotein from lymphocytes and absence of a glycoprotein Ib from platelets.

Clinical Features

A. Symptoms and Signs: Recurrent infection usually does not start until after 6 months of age. Patients are susceptible to infection with capsular polysaccharide-type organisms (eg, pneumococcus, meningococcus, and *H influenzae*) and may develop meningitis, otitis media, pneumonia, and sepsis. As they become older, they become susceptible to infection with other types of organisms and may have recurrent viral infection.

Eczema is usually present by 1 year of age and is typical in distribution. It may be associated with other allergic manifestations. Frequently, it is secondarily infected.

Thrombocytopenia is present at birth and may result in early manifestations of bleeding. Bleeding usually increases during episodes of infection and is associated with a decrease in the platelet count. The bleeding tendency becomes less severe as the child becomes older.

B. Laboratory Findings: Thrombocytopenia is present at birth and may assist in diagnosis. The platelet count may range from 5000 to 100,000/μL. Platelets are small in Wiskott-Aldrich syndrome, in contrast to other disorders associated with thrombocytopenia, eg, idiopathic thrombocytopenia, where they are increased in size. Anemia is frequently present and may be Coombs-positive. An increased incidence of chronic renal disease has been reported. Studies of B cell immunity demonstrate normal IgG, decreased IgM, increased IgA and IgE, low to absent isohemagglutinins, normal numbers of B cells, and an inability to respond to immunization with polysaccharide antigen. Paraproteins are frequently observed. T cell immunity is usually intact early in the disease but may decline with advancing years.

Immunologic Diagnosis

The earliest detected immunologic abnormality consists of hypercatabolism of immunoglobulins. The typical pattern of low to absent isohemagglutinins,

low IgM, and elevated IgA and IgE may not be present until 1 year of age. B cells are normal in number. Patients fail to form antibody following immunization with polysaccharide antigens. T cell immunity may be normal initially and show gradual attrition with advancing age.

Differential Diagnosis

When the complete syndrome is present, there is little confusion in diagnosis. Idiopathic thrombocytopenia in a male child may be difficult to differentiate from Wiskott-Aldrich syndrome. In idiopathic thrombocytopenia, the immunoglobulins, isohemagglutinins, and response to polysaccharide antigens are normal. Male patients with eczema and recurrent infection have normal immunologic studies and normal platelet counts, although they may have elevated IgA and IgE.

Treatment

Infections should be treated promptly and aggressively with antibiotics to cover the most common organisms. Corticosteroids should not be used to treat the thrombocytopenia, since they will enhance the susceptibility to infection. Splenectomy has been fatal in virtually all patients. Treatment of immunodeficiency is difficult. Infusions of frozen plasma have been utilized on a monthly basis as a source of passive protection. Intramuscular γ-globulin is not used because of the thrombocytopenia and potential bleeding at injection sites, but intravenous γ-globulin can be given (see treatment of hypogammaglobulinemia, above). Transfer factor has been advocated, but controlled studies have not been performed. Successful bone marrow transplantation has been performed.

Complications & Prognosis

With aggressive therapy, the long-term prognosis has improved. Immediate complications are related to bleeding episodes and acute infection. As patients become older, they become susceptible to a wider spectrum of microbial agents. Chronic keratitis secondary to viral infection is frequent. Lymphoreticular cancers, especially of the central nervous system, occur in older patients. Myelogenous leukemia occurs more frequently in this disorder than in other immunodeficiency disorders.

IMMUNODEFICIENCY WITH THYMOMA

Recurrent infection may be the presenting sign if the thymoma is associated with immunodeficiency. Infection takes the form of sinopulmonary infection, chronic diarrhea, dermatitis, septicemia, stomatitis, and urinary tract infection. Thymoma has also been associated with muscle weakness (when found in conjunction with myasthenia gravis), aregenerative anemia, thrombocytopenia, diabetes, amyloidosis, chronic hepatitis, and the development of nonthymic cancer.

Patients with acquired hypogammaglobulinemia should be followed at regular intervals for the development of thymoma, usually detected on routine chest x-rays. Occasionally, the thymoma may be detected prior to the development of immunodeficiency. Marked hypogammaglobulinemia is usually present. The antibody response following immunization may be abnormal. Some patients have deficient T cell immunity as assayed by delayed hypersensitivity skin tests and response of peripheral blood lymphocytes to PHA. Increased suppressor cell activity has been described in some patients. In patients who have aregenerative anemia, pure red cell aplasia is seen on marrow aspiration. Thrombocytopenia, granulocytopenia, and autoantibody formation are occasionally observed. In 75% of cases, the thymoma is of the spindle cell type. Some tumors may be malignant.

In no instance has the removal of the thymoma resulted in improvement of immunodeficiency. This is in contrast to pure red cell aplasia and myasthenia gravis, which may improve following removal of thymoma. γ-Globulin is of benefit in controlling recurrent infections and chronic diarrhea. (See treatment of hypogammaglobulinemia, above.)

The overall prognosis is poor, and death secondary to infection is common. Death may also be related to associated abnormalities such as thrombocytopenia and aregenerative anemia.

IMMUNODEFICIENCY WITH SHORT-LIMBED DWARFISM

Three forms of immunodeficiency with short-limbed dwarfism exist. Type I is associated with combined immunodeficiency, type II with T cell immunodeficiency, and type III with B cell immunodeficiency.

The clinical features of each type vary with the degree of immunodeficiency. In short-limbed dwarfism associated with combined immunodeficiency, symptoms of infection are identical to those seen in severe combined immunodeficiency disease. Susceptibility to viral, bacterial, fungal, and protozoal infection is observed. Patients usually die in the first year. Patients with short-limbed dwarfism and T cell immunodeficiency are susceptible to recurrent sinopulmonary infection, fatal varicella, and progressive vaccinia and may develop a malabsorptionlike syndrome. Patients with short-limbed dwarfism and B cell immunodeficiency experience recurrent pyogenic infections in the form of pneumonia, sepsis, otitis media, and meningitis. In all patients, short-limbed dwarfism is characterized by short, pudgy hands and extremities. The head is normal in size, which distinguishes this disorder from achondroplasia. During infancy, redundant skin folds are often seen around the neck and large joints of the extremities. Patients with short-limbed dwarfism and T cell immunodeficiency may

also have cartilage-hair hypoplasia manifested by light, thin, and sparse hair.

Abnormal immunologic studies vary with the degree of immunodeficiency. In short-limbed dwarfism associated with combined immunodeficiency, there is absence of T and B cell immunity. In short-limbed dwarfism associated with T cell immunodeficiency, T cell immunity is deficient as measured by delayed hypersensitivity skin tests and responsiveness of peripheral blood lymphocytes to PHA, allogeneic cells, and varicella antigens. B cell immunity is intact. In short-limbed dwarfism associated with B cell immunodeficiency, B cell immunity is absent and T cell immunity is intact.

Radiologic abnormalities consist of scalloping, irregular sclerosis, and cystic changes in the metaphyseal portions of long bones. Aganglionic megacolon has been reported. Patients with cartilage-hair hypoplasia have reduced hair diameters and lack the pigmented central core.

Treatment of these disorders is individualized to the associated immunodeficiency (eg, severe combined immunodeficiency, cellular immunodeficiency, and antibody immunodeficiency).

The prognosis varies with the degree of immunodeficiency. There have been no survivors with severe combined immunodeficiency. Patients with T cell immunodeficiency may survive to the fourth or fifth decade only to succumb to overwhelming varicella infection. The prognosis in patients with antibody immunodeficiency is similar to that of X-linked hypogammaglobulinemia, but loss of T cell function may occur with time.

IMMUNODEFICIENCY WITH ENZYME DEFICIENCY

Adenosine Deaminase & Nucleoside Phosphorylase Deficiency

Patients with enzyme deficiency and immunodeficiency may have clinical and laboratory abnormalities identical with those of patients with immunodeficiency with normal enzyme activity. Enzyme deficiency as a cause of immunodeficiency probably accounts for fewer than 15% of immunodeficiency disorders at present. It is almost certain that additional enzyme deficiencies will be discovered.

Adenosine deaminase and purine nucleoside phosphorylase are enzymes necessary for the normal catabolism of purines (Fig 20–1). Adenosine deaminase catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine. Nucleoside phosphorylase catalyzes the conversion of inosine, deoxyinosine, guanosine, and deoxyguanosine to hypoxanthine and guanine (Fig 20–1). Several mechanisms have been postulated to explain the means whereby these enzyme deficiencies result in immunodeficiency. Experimental evidence indicates that adenosine, in increased amounts, may result in increased cAMP activity, which is known to be associated with inhibition of lymphocyte function. Adenosine has also been shown to be toxic to cells in culture as a result of pyrimidine starvation. There is also evidence that exogenous adenosine can lead to the intracellular accumulation of S-adenosylhomocysteine, which acts as a potent inhibitor of DNA methylation. However, the most likely mechanism of inhibition of lymphocyte function is a result of the accumulation of deoxyadenosine and subsequently deoxyATP, which results in inhibition of ribonucleotide reductase and subsequent depletion of deoxyribonucleoside triphosphates. In purine nucleoside phosphorylase deficiency, deoxyguanosine has been shown to result in accumulation of deoxyGTP. Again, this most likely results in inhibition of ribonucleotide reductase. These mechanisms have great importance in devising potential biochemical treatment for these disorders.

The degree of combined immunodeficiency is variable. The spectrum of immunologic aberrations varies from complete absence of T and B cell immunity, as observed in patients with severe combined immunodeficiency disease, to mild abnormalities of T and B cell function. Patients with enzyme deficiencies should be evaluated completely to determine the extent of the immunologic deficiency. As a result of the marked variability in immunodeficiency, there is a considerable variation in the age at onset, severity of symptoms, and eventual outcome. Patients with adenosine deaminase deficiency and severe combined immunodeficiency may have radiologic abnormalities that include concavity and flaring of the anterior ribs, abnormal contour and articulation of posterior ribs and transverse processes, platyspondylisis, thick growth arrest lines, and an abnormal bony pelvis. Patients with nucleoside phosphorylase deficiency and T cell immunodeficiency have normal bone x-rays, absent T cell immunity, normal B cell immunity, a history of recurrent infection, and autoantibody formation. They are susceptible to fatal varicella and vaccinia virus infection.

The mode of inheritance of these enzyme defects appears to be autosomal recessive. The carrier state can be demonstrated in both sexes as evidenced by diminished adenosine deaminase or nucleoside phosphorylase activity. The enzymes are absent in red cells, white cells, tissue, and cultured fibroblasts in these patients. An intrauterine diagnosis of adenosine deaminase deficiency has been made. Patients may not be immunodeficient at birth.

Treatment of this disorder is similar to that of severe combined immunodeficiency or combined immunodeficiency. Several successful bone marrow transplants have been performed, with subsequent return of immunologic function. The patient's cells continue to have absent enzyme activity following transplantation.

Successful treatment of patients with adenosine deaminase deficiency has been reported utilizing monthly infusions of irradiated red blood cells as a source of the enzyme. Other patients have responded partially or not at all. Biochemical treatment of a sin-

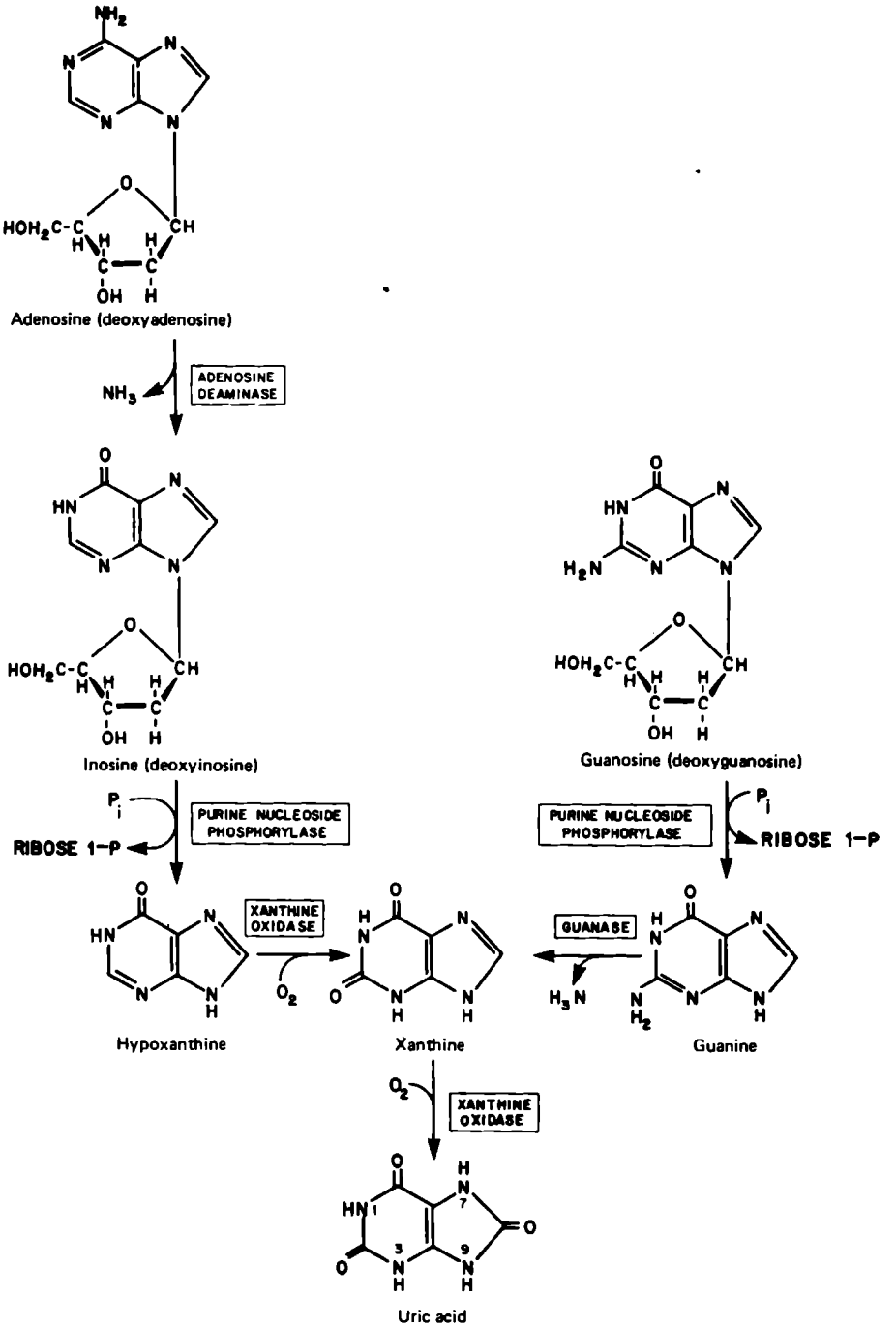

Figure 20–1. Generation of uric acid from purine deoxyribonucleosides by way of the purine bases hypoxanthine, xanthine, and guanine. (Reproduced, with permission, from Martin DW Jr et al: *Harper's Review of Biochemistry*, 20th ed. Lange, 1985.)

gle nucleoside phosphorylase-deficient patient with oral uridine was unsuccessful. Deoxycytidine therapy is currently under evaluation. Other patients have responded partially to thymosin or thymus transplantation (or both).

5'-Nucleotidase Deficiency

There have been several reports of decreased activity of 5'-nucleotidase and immunodeficiency. This deficiency has been described in association with acquired hypogammaglobulinemia, X-linked hypogammaglobulinemia, and selective IgA deficiency. However, 5'-nucleotidase may be a differentiation marker of lymphocytes—in particular B lymphocytes—and the deficiency therefore may reflect a diminished number of B cells or an abnormality of maturation in the peripheral circulation of these patients.

Transcobalamin II Deficiency

Several patients have been described with a deficiency of transcobalamin II, a vitamin B_{12}-binding protein necessary for the transport of vitamin B_{12} into cells. These patients were found to have hypogammaglobulinemia, macrocytic anemia, lymphopenia and granulocytopenia, thrombocytopenia, and severe intestinal malabsorption. Vitamin B_{12} treatment resulted in the reversal of all of the manifestations of the disorder. Specific antibody synthesis occurred following administration of vitamin B_{12}.

Biotin-Dependent Carboxylase Deficiencies

Patients have been described with infantile chronic mucocutaneous candidiasis, ataxia, alopecia, intermittent lactic acidosis, and increased excretion of β-hydroxypropionate, methylcitrate, β-methylcrotonylglycine, and 3-β-hydroxyisovalerate in the urine. Immunologic abnormalities were found in both B and T cell function. A second (neonatal) form has been described associated with severe acidosis and multiple episodes of sepsis. An intrauterine diagnosis has been made, and intrauterine therapy with biotin has been given. Treatment with biotin, 10 mg/d, reduced the abnormal metabolites in the urine and reversed the alopecia, ataxia, and chronic candidiasis. Multiple biotin-dependent carboxylase deficiencies may be one of several causes of the chronic mucocutaneous candidiasis syndrome with abnormal T cell function or severe recurrent sepsis.

GRAFT-VERSUS-HOST (GVH) DISEASE

GVH disease occurs when there is an unopposed attack of histo*in*compatible cells on an individual who is unable to reject foreign cells. The requirements for the GVH reaction are (1) histocompatibility differences between the graft (donor) and host (recipient), (2) immunocompetent graft cells, and (3) immunodeficient host cells. A GVH reaction may result from the infusion of any blood product containing viable lymphocytes, as may occur in maternal-fetal blood transfusion, intrauterine transfusion, therapeutic whole blood transfusions, or transfusions of packed red cells, frozen cells, platelets, fresh plasma, or leukocyte-poor red cells; or transplantation of fetal thymus, fetal liver, or bone marrow. The GVH reaction may have its onset 7–30 days following infusion of viable lymphocytes. Once the reaction is established, little can be done to modify its course. In the majority of immunodeficient patients, a GVH reaction is fatal. The exact mechanism whereby a GVH reaction is produced is not known. All cells of the body have histocompatibility antigens with the exception of red blood cells. Biopsy of active GVH lesions usually demonstrates infiltrations by mononuclear cells and eosinophils as well as phagocytic and histiocytic cells. The GVH reaction may appear in 3 distinct forms: acute, hyperacute, and chronic.

In the **acute form,** the initial manifestation is a maculopapular rash which is frequently mistaken for a viral or allergic rash. Initially, it blanches with pressure and then becomes diffuse. If the rash is persistent, it will begin to scale. Diarrhea, hepatosplenomegaly, jaundice, cardiac irregularity, central nervous system irritability, and pulmonary infiltrates may occur during the height of the reaction. Enhanced susceptibility to infection is also present and may result in death from sepsis.

In the **hyperacute form** of GVH reaction, the rash may also begin as a maculopapular lesion but then rapidly progresses to a form resembling toxic epidermal necrolysis, usually associated with severe diarrhea. This has not been associated with staphylococcal infection. Clinical and laboratory abnormalities similar to those found in the acute form may be observed. Death occurs shortly after the onset of the reaction.

The **chronic form** of GVH disease may be a result of maternal-fetal transfusion or attempts at immunotherapy with histocompatible bone marrow transplantation. The clinical and laboratory features may be markedly abnormal or only slightly so. Chronic desquamation of the skin is usually present. Hepatosplenomegaly may be prominent along with lymphadenopathy. Chronic diarrhea and failure to thrive are usually present. Secondary infection is a frequent complication. On biopsy of skin or lymph nodes, histiocytic infiltration may be found, leading to an erroneous diagnosis of Letterer-Siwe disease. Patients with Letterer-Siwe disease have normal immunoglobulin values and normal T cell immunity, but patients with chronic GVH disease have severe immunodeficiency. Chronic GVH disease has also been confused with acrodermatitis enteropathica.

The diagnosis is suggested by the diffuse clinical abnormalities present in a patient known to have cellular immunodeficiency and who has received a transfusion of potentially immunocompetent cells in the preceding 5–30 days. The diagnosis is established by the demonstration of sex chromosome or HLA chimerism. On occasion, patients with known GVH disease

fail to have detectable chimerism. Incubation of peripheral blood mononuclear cells with interleukin-2 may result in detectable chimerism.

There is no adequate treatment of GVH disease once it is established. Use of corticosteroids only enhances the susceptibility to infection. Antilymphocyte globulin also results in further suppression of immunity. Cyclosporine, an experimental immunosuppressive agent, may be useful. The only treatment is prevention. Any infant suspected of having cellular immunodeficiency who requires the administration of a blood product should receive cells that have been subjected to 3000–6000 R of radiation to destroy viable lymphocytes and thus prevent GVH disease. Blood products to be irradiated include whole blood, packed red blood cells, leukocyte-poor red blood cells, and fresh plasma.

IMMUNODEFICIENCY WITH CELL MEMBRANE ABNORMALITIES

A number of immunodeficiency disorders have now been linked to deficiencies of an essential cell membrane component. Undoubtedly, more of these abnormalities will be discovered. The 3 primary syndromes that have been described include the bare lymphocyte syndrome, LFA-1/Mac-1 glycoprotein deficiency, and gpL-115 glycoprotein deficiency.

Bare Lymphocyte Syndrome

Patients with the bare lymphocyte syndrome have a deficient expression of HLA antigens associated with immunodeficiency. β_2-Microglobulin, a structural component of HLA antigens, is not found on the surface of cells and results in inability to detect HLA-A, -B, or -C determinants. Several patients have been reported with a deficiency of Dr determinants. Abnormalities in affected patients vary considerably. Some individuals have been entirely healthy, whereas others have developed aplastic anemia, opportunistic infection, chronic diarrhea, recurrent infection, and varying degrees of immunodeficiency. Symptomatic patients are lymphopenic and have decreased T cell numbers with normal or elevated B cell numbers. The in vitro response to mitogens and allogeneic cells is usually normal, whereas the response to antigens is absent.

LFA-1/Mac-1 Glycoprotein Deficiency

LFA-1/Mac-1 glycoprotein deficiency is associated with recurring bacterial infection, granulocytosis, impaired inflammatory response, and delayed umbilical cord separation. Granulocytes and monocytes function abnormally with decreased adherence and phagocytosis of opsonized particles, decreased chemotaxis, and decreased superoxide generation. Granulocyte and monocyte surface expression of these glycoproteins, termed LFA-1 and Mac-1, is deficient. Similar abnormalities have been demonstrated in patient lymphocytes associated with abnormal lymphocyte responses to mitogens and EBV. Deficient NK cell activity has also been described.

Deficient gpL-115 Membrane Glycoprotein

Several patients have been described who had a clinical syndrome of recurrent viral, protozoal, and bacterial infections. These patients had normal levels of serum immunoglobulins. A decreased response of lymphocytes to mitogens and specific antigens was described. Lymphocytes were also characterized as having a reduced cell volume.

PHAGOCYTIC DYSFUNCTION DISEASES

Phagocytic disorders may be divided into extrinsic and intrinsic defects. Included in the extrinsic category are deficiencies of opsonins secondary to deficiencies of antibody and complement factors; suppression of the total number of phagocytic cells by immunosuppressive agents; interference of phagocytic function by corticosteroids; and suppression of the number of circulating neutrophils by autoantibody directed specifically against neutrophil antigens. Other extrinsic disorders may be related to abnormal neutrophil chemotaxis secondary to complement deficiency or abnormal complement components. Intrinsic phagocytic disorders are related to enzymatic deficiencies within the metabolic pathway necessary for killing of bacteria. These include chronic granulomatous disease with a deficiency of NADPH or NADH oxidase, myeloperoxidase deficiency, and glucose-6-phosphate dehydrogenase deficiency.

Susceptibility to infection in phagocytic dysfunction syndromes may range from mild recurrent skin infections to severe overwhelming, fatal systemic infection. Characteristically, all of these patients are susceptible to bacterial infection and have little difficulty with viral or protozoal infections. Some of the more severe disorders may be associated with overwhelming fungal infections.

Numerous tests can now be performed to evaluate phagocytic dysfunction (see Chapter 18). Screening tests are listed in Table 20–3 and definitive studies in Table 20–7.

CHRONIC GRANULOMATOUS DISEASE

Major Immunologic Features

- Susceptibility to infection with unusual organisms normally of low virulence, eg, *Staphylococcus epidermidis, Serratia marcescens, Aspergillus.*
- X-linked inheritance (autosomal variant occurs).
- Onset of symptoms by 2 years of age: draining

lymphadenitis, hepatosplenomegaly, pneumonia, osteomyelitis, abscesses.

- Diagnosis established by quantitative nitroblue tetrazolium test, quantitative killing curve, or chemiluminescence.

General Considerations

Chronic granulomatous disease is inherited as an X-linked disorder with clinical manifestations appearing during the first 2 years of life. An autosomal variant of the disease has been described. Patients are susceptible to infection with a variety of normally nonpathogenic and unusual organisms. Characteristic abnormal laboratory studies will detect both patients and female carriers of the disease. Female carriers are usually asymptomatic. Early diagnosis and aggressive therapy have improved the prognosis in these patients.

There are several different genetic forms of chronic granulomatous disease based on differing biochemical abnormalities. Absence of cytochrome b has been described in the X-linked form. Absence of cytochrome b has also been reported in females, suggesting an autosomal inheritance in some instances. The disease may also result from a low-affinity NADPH oxidase. In other variants, glutathione peroxidase or flavoprotein component is felt to be deficient. As a result of these enzymatic deficiencies, the intracellular metabolism of neutrophils and monocytes is abnormal, resulting in decreased oxygen consumption, decreased utilization of glucose by the hexose monophosphate shunt, decreased production of hydrogen peroxide, diminished iodination of bacteria, and decreased superoxide anion production. The net result is decreased intracellular killing of certain bacteria and fungi.

Clinical Features

A. Symptoms and Signs: In the majority of patients, the diagnosis can be established before 2 years of age. The most frequent abnormalities consist of marked lymphadenopathy, hepatosplenomegaly, chronic draining lymph nodes, and at least one episode of pneumonia. Other symptoms include rhinitis, conjunctivitis, dermatitis, ulcerative stomatitis, perianal abscess, osteomyelitis, chronic diarrhea with intermittent abdominal pains, and intestinal obstruction. Chronic and acute infection occurs in lymph nodes, skin, lung, intestinal tract, liver, and bone. A major clue to early diagnosis is the finding of a normally nonpathogenic or unusual organism. Organisms responsible for infection include *S aureus, S epidermidis, S marcescens, Pseudomonas, Escherichia coli, Candida,* and *Aspergillus*.

Table 20–7. Evaluation of phagocytosis.

Test	Comment
Quantitative nitroblue tetrazolium (NBT)	Used for diagnosis of chronic granulomatous disease and for detection of carrier state.
Quantitative intracellular killing curve	Used for diagnosis of chronic granulomatous disease. Can be performed using organisms isolated from individual.
Chemotaxis	Abnormal in a variety of disorders associated with frequent bacterial infection. Does not provide a specific diagnosis. Performed using a Boyden chamber utilizing a microscopic or radioactive technique. Rebuck skin window provides a qualitative result in vivo.
Random migration	Abnormal in "lazy leukocyte syndrome." Tests nonchemotactic migration of leukocytes.
Chemiluminescence	Abnormal in chronic granulomatous disease and myeloperoxidase deficiency.

B. Laboratory Findings: The most readily available study for diagnosis is the quantitative nitroblue tetrazolium test. (See Chapter 18.) Patients have absent nitroblue tetrazolium dye reduction, whereas carriers may have normal or reduced nitroblue tetrazolium reduction. Patients with chronic granulomatous disease are unable to kill certain bacteria at a normal rate. The killing curves for organisms to which these individuals are susceptible usually indicate little or no killing in a period of 2 hours. Other abnormal findings include decreased oxygen uptake during phagocytosis and abnormal bacterial iodination. NK cell activity is normal.

The peripheral white cell count is usually elevated even if the patient does not have active infection. Hypergammaglobulinemia is present, and antibody function is normal. Cell-mediated immunity is normal, and complement factors may be elevated. During episodes of pneumonia, the chest x-ray is frequently severely abnormal. Liver function tests may be abnormal as a result of chronic infection. Pulmonary function tests are usually abnormal following episodes of pneumonia and may not return to normal for several months. Several patients have been reported to have a rare Kell blood group, the "McLeod" phenotype.

Immunologic Diagnosis

A diagnosis can be established utilizing the quantitative nitroblue tetrazolium dye reduction assay or quantitative chemiluminescence. Confirmation is obtained utilizing specific bactericidal assays. These assays may also be utilized to identify the carrier state and to establish an intrauterine diagnosis. Both male and autosomal variants of chronic granulomatous disease have abnormal studies. B cell immunity, T cell immunity, and complement are normal. Chemiluminescence is the best method for detecting the carrier state. An intrauterine diagnosis has been made using fetal blood and a nitroblue tetrazolium test.

Differential Diagnosis

Few clinical disorders are easily confused with chronic granulomatous disease. Two other disorders with abnormal enzymatic function are associated with clinical symptoms and laboratory features similar to those of chronic granulomatous disease. One of these is the autosomal variant of chronic granulomatous disease associated with deficient glutathione peroxidase.

the other is associated with deficient glucose-6-phosphate dehydrogenase. Any child presenting with osteomyelitis, pneumonia, liver abscess, or chronic draining lymphadenopathy associated with a normally nonpathogenic or unusual organism should be suspected of having chronic granulomatous disease.

Treatment

Aggressive diagnostic measures and therapy are necessary for long-term survival and diminished morbidity. Blood cultures, aspiration of draining lymph nodes, liver biopsy, and open lung biopsy should be utilized to obtain a specific bacterial diagnosis. Therapy should be instituted immediately while results of cultures are pending. The choice of antibiotics should be one that covers the spectrum of infectious agents. An appropriate choice would be penicillin and gentamicin or penicillin and chloramphenicol. These agents cover the majority of organisms with the exception of *Candida* and *Aspergillus*. For these latter organisms, amphotericin is the treatment of choice. Amphotericin therapy should be given intravenously, starting with high doses in the range of 1 mg/kg/d. The ultimate survival of the patient is dependent upon early and intensive therapy. Treatment of the patient with antibiotics may be prolonged, requiring 5–6 weeks of total therapy. Additional therapy has included the use of white blood cell infusions, but experience has been extremely limited. Several investigators have utilized continuous anti-infective therapy with sulfisoxazole. A single successful bone marrow transplant has been performed.

Complications & Prognosis

Chronic organ dysfunction may result from severe or chronic infection. Examples are abnormal pulmonary function, chronic liver disease, chronic osteomyelitis, and malabsorption secondary to gastrointestinal tract involvement. The mortality rate in chronic granulomatous disease has been considerably reduced by early diagnosis and aggressive therapy. Survivals into the second decade and beyond have been recorded. Female carriers have an increased incidence of systemic and discoid lupus erythematosus.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

Glucose-6-phosphate dehydrogenase deficiency is inherited in an X-linked manner. Complete absence of leukocyte glucose-6-phosphate dehydrogenase activity has been associated with a clinical picture similar to that of chronic granulomatous disease. The defect in white blood cells is believed to be a result of deficient generation of NADPH needed as a reducing equivalent for the oxidase. Some investigators have demonstrated decreased hexose monophosphate shunt activity and decreased hydrogen peroxide production in the white blood cells. Leukocytes are unable to kill certain organisms at a normal rate in a manner similar to that found in chronic granulomatous disease. The susceptibility of these patients to microbial agents is similar to that of patients with chronic granulomatous disease. Glucose-6-phosphate dehydrogenase deficiency differs in that the onset is later, both males and females are affected, and hemolytic anemia is present. The laboratory diagnosis is based on the demonstration of deficient white blood cell glucose-6-phosphate dehydrogenase. The nitroblue tetrazolium test, the killing curve, the production of hydrogen peroxide, and oxygen consumption are abnormal. Treatment and prognosis are similar to those of chronic granulomatous disease.

MYELOPEROXIDASE DEFICIENCY

Several patients with complete deficiency of leukocyte myeloperoxidase have been described. Myeloperoxidase is one of the enzymes necessary for normal intracellular killing of certain organisms. The leukocytes of these patients have normal oxygen consumption, hexose monophosphate shunt activity, and superoxide and hydrogen peroxide production. The intracellular killing of organisms is delayed but may reach normal levels with increased incubation times. Chemiluminescence of white blood cells is decreased. Susceptibility to candidal and staphylococcal infections has been the chief problem. The diagnosis can be established utilizing a peroxidase stain of peripheral blood. No specific treatment is available other than appropriate antibiotic therapy.

ALKALINE PHOSPHATASE DEFICIENCY

Several patients have been reported who have recurrent bacterial infection associated with absent leukocyte alkaline phosphatase activity. There is a modest reduction in bactericidal activity.

CHEDIAK-HIGASHI SYNDROME

Chédiak-Higashi syndrome is a multisystem autosomal recessive disorder. Symptoms include recurrent bacterial infections with a variety of organisms, hepatosplenomegaly, partial albinism, central nervous system abnormalities, and a high incidence of lymphoreticular cancers.

The characteristic abnormality of giant cytoplasmic granular inclusions in white blood cells and platelets is observed on routine peripheral blood smears under ordinary light microscopy. Additional abnormalities include elevated EBV antibody titers, abnormal neutrophil chemotaxis, decreased NK cell activity, and abnormal intracellular killing of organisms (including streptococci and pneumococci as well as those organisms found in chronic granulomatous

disease). The killing defect consists of delayed killing time. Oxygen consumption, hydrogen peroxide formation, and hexose monophosphate shunt activity are normal. Abnormal microtubule function, abnormal lysosomal enzyme levels in granulocytes, and protease deficiency in granulocytes have been described and are associated with increased levels of leukocyte cAMP. Correction of abnormal leukocyte function in vitro has been accomplished utilizing ascorbate, but the results of treatment in vivo are contradictory. Improved granulocyte function in vitro has also been observed using anticholinergic agents.

There is no treatment other than specific antibiotic therapy for infecting organisms. The prognosis is poor because of increasing susceptibility to infection and progressive neurologic deterioration. Most patients die during childhood, but survivors to the second and third decade have been reported.

JOB'S SYNDROME

Job's syndrome was originally described as a disorder of recurrent "cold" staphylococcal abscesses of the skin, lymph nodes, or subcutaneous tissue. The first patients were fair-skinned, red-headed girls of Italian descent. Initial descriptions also included eczematoid skin lesions, otitis media, and chronic nasal discharge. Few signs of systemic infection or inflammatory response occurred in association with the infection. Additional reports of Job's syndrome indicated that the disorder might be a variant of chronic granulomatous disease. However, most of the patients studied do not have abnormal immunologic tests. Patients with hyper-IgE syndrome have clinical and laboratory features similar to Job's syndrome; in fact, these may be the same disorder. (See Hyper-IgE Syndrome, below.)

Treatment consists of appropriate antibiotic therapy. The prognosis is uncertain.

TUFTSIN DEFICIENCY

Tuftsin deficiency has been reported as a familial deficiency of a phagocytosis-stimulating tetrapeptide that is cleaved from a parent immunoglobulin (termed leukokinin) molecule in the spleen. Tuftsin also appears to be absent in patients who have been splenectomized. Local and severe systemic infections occur. Organisms include *Candida, S aureus,* and *S pneumoniae*. Tuftsin levels are determined only in a few specialized laboratories.

There is no treatment, and the prognosis is uncertain. γ-Globulin therapy appeared to be beneficial in the 2 families reported.

LAZY LEUKOCYTE SYNDROME

Patients have been described who have a defective chemotactic response of neutrophils in association with neutropenia. These individuals also have an abnormal in vivo inflammatory response as determined by the "Rebuck window," and they fail to demonstrate an increased number of peripheral blood neutrophils following epinephrine or endotoxin stimulation. The random migration of peripheral leukocytes is abnormal as determined by the vertical migration of white blood cells in a capillary tube. Patients are susceptible to severe bacterial infections.

Treatment with specific antibiotics is indicated. The prognosis is unknown.

ELEVATED IgE, DEFECTIVE CHEMOTAXIS, ECZEMA, & RECURRENT INFECTION (Hyper-IgE Syndrome)

These patients—both males and females—have an early onset of eczema and recurrent bacterial infections in the form of abscesses. Structures involved include the skin, lungs, ears, sinuses, and eyes. Systemic infection may involve other areas. Organisms causing infection include *S aureus, Candida, H influenzae, S pneumoniae,* and group A streptococci. Laboratory findings consist of eosinophilia, IgE concentrations in excess of 5000 IU/mL, diminished antibody response following immunization, and normal lymphocyte response to PHA and Con A but reduced response to antigens and allogeneic cells (MLC). Abnormalities of chemotaxis are present in some but not all patients. In vitro, patients have been shown to have decreased suppressor T cells with increased spontaneous IgE production. Increased amounts of IgE antibody to staphylococcal antigens are found. Antibiotic therapy is indicated for specific infections. The prognosis is unknown, although patients have survived to adulthood.

LEUKOCYTE MOVEMENT DISORDERS

A number of patients have been described with decreased leukocyte chemotaxis and recurrent infections (usually bacterial). In some, deficiency of IgG and an IgG inhibitor of chemotaxis have also been found. Defective actin polymerization was found in association with defective neutrophil phagocytosis and locomotion. Abnormal chemotaxis has been found in congenital ichthyosis. Mannosidosis, a storage disease manifested by mental retardation and recurrent infections, is associated with abnormal chemotaxis and delayed phagocytosis. Mannose, which accumulates within leukocytes, may interfere with cell function. Similar abnormalities have been described in type IB glycogen storage disease.

MISCELLANEOUS PHAGOCYTIC DISORDERS

A variety of rare phagocytic disorders have been described. They are usually associated with recurrent

skin infections and systemic bacterial infections. Diagnosis of these rare disorders is usually made with sophisticated tests not available in most medical centers. Abnormalities that have been described include decreased lactoferrin granules, abnormal polymerization of actin, deficiency of specific granules, and absent neutrophil surface membrane glycoprotein. Because of the limited number of patients, recommendations regarding treatment and prognosis cannot be made.

COMPLEMENT ABNORMALITIES & IMMUNODEFICIENCY DISEASES

A variety of complement deficiencies and abnormalities of complement function have been associated with increased susceptibility to infection (Table 20–8). Complement factors are necessary for normal opsonization, bacterial killing, and neutrophil chemotaxis. Despite the participation of complement components in the phagocytic process, a number of complement deficiences are unassociated with enhanced susceptibility to infection. Many of these disorders are associated with increased susceptibility to autoimmune disease (see Chapter 21). Hereditary angioneurotic edema as a result of deficiency of C1 esterase inhibitor is discussed elsewhere (see Chapter 24).

C1q DEFICIENCY

A deficiency of C1q has been reported in patients with X-linked hypogammaglobulinemia and severe combined immunodeficiency disease. The cause of this deficiency is not certain, but it may be related to hypercatabolism as a result of enhanced susceptibility to infection in patients with primary immunodeficiency disorders. The degree to which C1q deficiency increases the susceptibility to infection in patients with other primary immunodeficiency disorders is not known. C1q deficiency has also been described in patients with an SLE-like syndrome and increased susceptibility to bacterial infection. C1q deficiency has also been reported in patients with urticarialike lesions and cutaneous vasculitis, sometimes associated with precipitins to C1q.

Table 20–8. Evaluation of complement disorders.

C1q, C1r, C1s	Deficiency initially suspected by decreased hemolytic complement (CH_{50}). Specific assays required for confirmation.
C2, C4, C5, C6, C7, C8, C9	Deficiency initially suspected by decreased CH_{50}. Specific assays required for confirmation.
C3, C4	Deficiency detected using quantitative assay available in most hospital laboratories.
C5 dysfunction	Present in normal amounts but abnormal in function in "C5 dysfunction" syndrome.

C1r & C1s DEFICIENCY

Familial deficiencies of C1r and C1s have been described in patients with susceptibility to autoimmune disease. Most of these patients had an SLE-like syndrome. In addition, these patients appeared to have an increased susceptibility to bacterial infection. No specific treatment of this disorder has been proposed. The infusion of complement components might theoretically result in enhanced immune complex disease and worsening of the SLE-like disorder.

C2 DEFICIENCY

C2 deficiency has been reported in several patients with SLE-like disorders, anaphylactoid purpura, dermatomyositis, and increased susceptibility to infection. The patients have chronic renal disease and antibody directed against DNA. An autosomal recessive mode of inheritance is suggested by familial studies. The patients are susceptible to bacterial infection. Recently, C2 deficiency has been associated with the HLA haplotype A10, B18 and has also been found in association with hypogammaglobulinemia.

Treatment is directed toward the underlying autoimmune disease, with specific antibiotic therapy for infections. In one patient who received a blood transfusion, the levels of serum C3, C5, C6, and C7 decreased dramatically. This observation suggests that complement replacement therapy might result in increased activation of the complement components and increased immune complex disease.

C3 DEFICIENCY

Two forms of C3 deficiency exist. In type I, a marked decrease of C3 is present in the serum. However, C3 is probably deficient as a result of a deficiency of C3 inactivator. In the single patient reported, increased susceptibility to bacterial infection was present throughout life. The patient also had Klinefelter's syndrome. Infusion of normal plasma corrected the abnormalities in the patient and resulted in diminished susceptibility to infection.

A second form of C3 deficiency (type II) has been reported in several patients. Partial lipodystrophy was present in association with an increased susceptibility to bacterial infection and nephritis. The decreased level of C3 was found to be associated with increased destruction and decreased synthesis. The abnormalities were partially explained by the demonstration of an enzyme, C3 convertase, capable of cleaving C3 in vitro and in vivo.

C4 DEFICIENCY

C4 deficiency has been described in several individuals who were asymptomatic. The deficiencies

were detected in routine screening of normal blood donors. In another family, C4 deficiency was associated with an SLE-like syndrome. Linkage to the histocompatibility antigens A2, B12, and D2 (maternal) and A2, Bw15, and LD108 (paternal) has been noted. C4-deficient patients have diminished chemotactic and opsonic activity and impaired antibody responses.

FAMILIAL C5 DYSFUNCTION & C5 DEFICIENCY

C5 dysfunction has been described as a familial defect in patients presenting with failure to thrive, diarrhea, seborrheic dermatitis, and susceptibility to infection with bacterial organisms.

Laboratory studies usually demonstrate leukocytosis and hypergammaglobulinemia. The total hemolytic complement and C5 levels are normal. Despite the normal levels of C5, chemotaxis is ineffective and can be corrected by adding normal C5 to the testing procedure.

Treatment consists of appropriate antibiotic therapy for infecting organisms. Fresh plasma has been recommended as a potential source of normal C5. Fresh-frozen plasma is not believed to contain sufficient amounts for therapy. However, some of the clinical findings in patients with familial C5 dysfunction closely resemble those found in patients with cellular immunodeficiency disorders. The use of fresh irradiated plasma is recommended to prevent a GVH reaction if the diagnosis has not been established with certainty. Ideally, patients should have studies of T cell immunity performed prior to the institution of treatment.

C5 deficiency has been described in a child with SLE. C5 levels in other family members were either normal or 50% of normal.

C6 DEFICIENCY

Several patients with C6 deficiency have been described. In one case, parents of the patient and 5 of 6 siblings had C6 values that were 50% of normal. A male child with repeated episodes of meningococcal meningitis and C6 deficiency has been described. The parents had C6 levels that were 50% of normal. In others, either recurrent meningococcal or gonococcal bacteremia was present.

C7 DEFICIENCY

Several patients with C7 deficiency have been described. Associated diseases include Raynaud's phenomenon, sclerodactyly, telangiectasia, and ankylosing spondylitis. Partial deficiency of C7 was found in the patient's parents and children, suggesting an autosomal inheritance. Increased susceptibility to meningococcal and gonococcal infections was also noted.

C8 DEFICIENCY

Patients with deficiency of C8 and prolonged disseminated gonococcal and meningococcal infections have been described. One patient lacked both functional and immunochemical presence of C8, but no inhibitors for C8 could be identified. Bactericidal activity against *Neisseria gonorrhoeae* was absent from her serum but could be completely restored by the addition of purified C8. Earlier complement functions such as opsonization and chemotactic factor generation were normal. A family study suggested an autosomal codominant mode of inheritance for this disorder. Another family has been reported with C8 deficiency and xeroderma pigmentosum.

C9 DEFICIENCY

Several patients have been described with C9 deficiency. Hemolytic complement activity is reduced but not absent. Patients have not had clinical abnormalities.

ACQUIRED & SECONDARY IMMUNODEFICIENCY

A variety of disorders are associated with secondary immunodeficiency (Table 20–9). In some, the immunodeficiencies may be transient and may become normal with adequate treatment of the primary disease, eg, tuberculosis, leprosy. In others, the immunodeficiency may be permanent, eg, congenital rubella. Many of the secondary immunodeficiencies may be due to multiple factors. Those observed in cancer may be secondary to the tumor, associated with therapy, or secondary to malnutrition. The importance of secondary immunodeficiency is in the recognition that such states exist and result in enhanced susceptibility to opportunistic infection. In the future, the recognition of secondary immunodeficiency may assume greater importance as newer forms of immunologic reconstruction become available.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Major Immunologic Features

- Reduced helper/suppressor T cell ratios.
- Reduced peripheral blood lymphocyte response to mitogens and antigens.
- Elevated immunoglobulin levels.
- Reduced to absent antibody response following immunization.
- Increased circulating immune complexes.
- Reduced NK cell activity.
- Reduced interleukin-2 production.

Table 20–9. Secondary immunodeficiency.

Clinical Setting	T Cell	B Cell	Phagocytosis	Complement	Comments
Infection					
Rubella (congenital)	May have decreased T cells, PHA, MLC.	May have hypogammaglobulinemia or selective immunoglobulin deficiencies; no response to rubella immunization; decreased response to multiple antigens.	Normal.	Normal.	Defects vary with severity of disease.
Measles	Transient suppression of delayed hypersensitivity; decreased PHA.	Normal immunoglobulins; normal antibody response.	Normal.	Normal.	Similar effect may be seen with measles immunization.
Leprosy	Decreased delayed hypersensitivity; decreased response to *M leprae;* decreased PHA, T cells.	Decreased B cells in some, increased in others; increased antibody.	Unknown.	Unknown.	Immunologic deficiency greater in lepromatous form.
Tuberculosis	Decreased delayed hypersensitivity; decreased T cells; decreased MIF.	Immunoglobulins normal.	Unknown.	Unknown.	Severe infection may be associated with anergy.
Coccidioidomycosis	Decreased delayed hypersensitivity; lymphocyte blastogenesis, MIF to coccidioidal antigen.	Normal.	Unknown.	Unknown.	Usually specific decreased immunity. Generalized depression may be present.
Chronic infection	Usually normal.	Increased immunoglobulins.	Decreased chemotaxis.	Increased components.	Increased autoantibody.
Acute viral infection	Lymphopenia; decreased T cells; decreased PHA in some; depressed helper/suppressor cell ratio.	Normal.	Normal.	Normal.	Defect may vary with severity of illness.
Cytomegalovirus	Specific unresponsiveness to cytomegalovirus.	Elevated IgM, IgA.	Unknown.	Unknown.	
Multiple or repeated viral infection	Decreased T cells, PHA, MLC, helper cells.	Elevated immunoglobulins, IgA, antibody to virus.	Unknown.	Unknown.	Occurs in selected homosexual individuals. Cause unknown.
Malignant neoplastic disease					
Hodgkin's disease	Suppression of delayed hypersensitivity; decreased PHA; serum factors suppress T cells.	Immunoglobulins normal to increased; decreased antibody response to certain antigens.	Frequent pneumococcal and *H influenzae* infection; decreased chemotaxis.	Unknown.	Some abnormalities may be due to treatment or splenectomy.
Acute leukemia	Decreased delayed hypersensitivity, PHA.	Variable immunoglobulin levels.	Normal.	Unknown.	Some abnormalities due to treatment.
Chronic leukemia	Serum factors inhibit PHA.	Variable immunoglobulin levels.	Normal.	Unknown.	Some abnormalities due to treatment.
Nonlymphoid cancer	Variable decrease in delayed hypersensitivity; suppression of PHA, MLC, T cells; immunosuppressive factors.	Variable immunoglobulin levels.	Normal.	Some tumors associated with decreased components.	Some abnormalities of T cells related to severity of disease, others to immunosuppression or irradiation.
Myeloma	Increased suppressor T cells (? macrophages).	Impaired antibody response; decreased immunoglobulins.	Normal.	Decreased complement components.	
Autoimmune disease					
Systemic lupus erythematosus (SLE)	Decreased delayed hypersensitivity; decreased T cells, PHA, MLC; decreased suppressor cells in animal models and in humans.	Immunoglobulins usually elevated; increased antibody titers to multiple antigens.	Normal.	Certain congenital complement deficiencies (C1q, C1r, C1s, etc) associated with SLE; secondary complement deficiencies frequent.	Some T cell defects may be secondary to treatment.

PHA = phytohemagglutinin stimulation of lymphocytes; MLC = allogeneic cell stimulation of lymphocytes.

Table 20–9 (cont'd). Secondary immunodeficiency.

Clinical Setting	T Cell	B Cell	Phagocytosis	Complement	Comments
Autoimmune disease (cont'd)					
Rheumatoid arthritis	Decreased delayed hypersensitivity; decreased PHA, MLC.	Immunoglobulin levels usually increased; normal antibody response to antigens.	Normal.	Increase in complement levels.	Some patients with hypogammaglobulinemia have arthritis.
Chronic active hepatitis	Decreased delayed hypersensitivity; decreased lymphocyte cytotoxicity; decreased T cells; mitogen response normal to decreased.	Immunoglobulins increased.	Unknown.	Decreased values in some patients.	Steroids increase some abnormalities.
Protein-losing states					
Nephrotic syndrome	Normal.	Decreased IgG; IgM and IgA may be decreased; antibody response decreased.	Unknown.	Normal in idiopathic lipoid nephrosis, may be decreased in other forms.	
Protein-losing enteropathy	Decreased delayed hypersensitivity; decreased T cells, PHA, MLC.	Hypogammaglobulinemia frequent.	Unknown.	Unknown.	
Other disorders					
Diabetes	Decreased PHA; MLC normal.	Normal.	Decreased chemotaxis; poor bacterial ingestion.	Unknown.	
Alcoholic cirrhosis	Decreased PHA.	Unknown.	Abnormal chemotaxis.	Some components decreased.	
Malnutrition	Lymphopenia; decreased T cells; decreased delayed hypersensitivity.	Immunoglobulins normal; normal antibody response.	Abnormal bacterial killing.	Decreased CH_{50}.	
Burns	Decreased delayed hypersensitivity; lymphopenia.	Decrease in all immunoglobulins; normal antibody response.	Decreased phagocytic function; decreased chemotaxis.		
Sarcoidosis	Decreased delayed hypersensitivity; decreased PHA; inhibitory plasma factor.	Increased immunoglobulins; normal antibody response.	Unknown.	Unknown.	
Splenectomy	Normal.	Immunoglobulins normal; decreased antibody response to whole organisms; normal antibody response to purified antigens.	Normal.	Normal.	Tuftsin deficiency found in some patients.
Sickle cell disease	Normal in most; some have mild T cell impairment.	IgM may be decreased; decreased antibody response to whole organisms with normal response to purified antigens.	Decreased phagocytosis; decreased opsonization; defect in properdin.	Some defects in alternative pathway described.	Some immunologic abnormalities may be related to zinc deficiency.
Uremia	Decreased delayed hypersensitivity; serum blocking factors suppress PHA, MLC.	Immunoglobulins normal; normal antibody response.	Normal.	Levels may be reduced in certain diseases.	
Aging	Decreased delayed hypersensitivity; decreased mitogen response; decreased T cells; suppressor T cells variably abnormal.	Increased IgG (IgA in some); increased B cells; decreased IgG response to certain antigens.	Unknown.	Unknown.	Increased autoantibodies.
Subacute sclerosing panencephalitis	Specific unresponsiveness to measles antigen; blocking factor present in some.	Increased antibody to measles.	Unknown.	Unknown.	

PHA = phytohemagglutinin stimulation of lymphocytes; MLC = allogeneic cell stimulation of lymphocytes.

Table 20–9 (cont'd). Secondary immunodeficiency.

Clinical Setting	T Cell	B Cell	Phagocytosis	Complement	Comments
Other disorders (cont'd)					
Down's syndrome	Decreased lymphocyte response to PHA.	Impaired primary and secondary antibody responses.	Unknown.	Unknown.	Increased susceptibility to infection.
Newborns and premature infants	Increased suppressor cells.	Diminished IgM IgA; impaired ability to form antibody to a variety of antigens.	Decreased killing.	Decreased complement factors; abnormal chemotaxis.	Decreased placental transfer of IgG in prematures.
Immunosuppressive treatment					
Corticosteroids	Transient decrease due to sequestration.	Transient decrease; late: reduced immunoglobulin synthesis.	Inhibits release of lysosomal enzymes, decreases phagocytosis of IgG-coated particles.	No effect.	Actions of steroids differ in humans and mice.
Cytotoxic drugs (alkylating agents, antimetabolites)	Variable decrease in numbers and functions; responses suppressed or enhanced.	Variable decrease in numbers and function (primary antibody responses impaired).	Decreased production of neutrophils and monocytes.	No effect.	Effects depend upon multiple factors (see Chapter 16).
Antithymocyte globulin	Decrease in T cell numbers and functions.	Unknown (some T cell-dependent reactions are impaired).	Unknown.	Unknown.	Activity against other cells (eg, platelets).
Radiation	Decrease in T cell numbers and functions (may be prolonged).	Impaired antibody production.	Transient decrease in blood monocytes.	Unknown.	Total nodal irradiation produces long-lasting immunosuppression.
Cyclosporine	No change in T cell number; profound depression of allograft rejection reaction.	Inhibition of T cell-dependent antibody responses.	Unknown.	Unknown.	
Phenytoin, penicillamine	Unknown.	IgA deficiency, hypogammaglobulinemia.	Unknown.	Unknown.	May or may not be reversible.
Anesthesia	Inhibits function.	Inconclusive.	Reduced phagocytosis.	Unknown.	Effect may last for weeks.

PHA = phytohemagglutinin stimulation of lymphocytes; MLC = allogeneic cell stimulation of lymphocytes.

General Considerations

AIDS was recognized as early as 1979. The number of cases reported to the Centers for Disease Control (CDC) increased dramatically each year since then, and in 1982 the CDC declared AIDS a new epidemic. The total number of cases now exceeds 40,000. The CDC has defined AIDS as "T cell immunodeficiency in a previously healthy adult in association with opportunistic infection or Kaposi's sarcoma." This initial definition is now considered too restrictive, since it is apparent that many AIDS-related disorders exist, eg, other types of cancer and lymphadenopathy syndrome. This has been termed the AIDS-related complex (ARC). The major clinical forms of AIDS include opportunistic infection, Kaposi's sarcoma, other cancers, and lymphadenopathy syndrome. These forms of AIDS are not mutually exclusive; eg, patients with opportunistic infection or lymphadenopathy may develop Kaposi's sarcoma. A diagnosis of AIDS must be made with caution, since there are widespread psychologic, social, and public health considerations associated with each reported case. The number of individuals who have antibody to the retrovirus associated with AIDS but who are asymptomatic is estimated to be in excess of 2 million in the USA.

Immunologic Pathogenesis

The etiologic agent that has been most closely associated with AIDS is a retrovirus termed human T cell lymphotropic virus/lymphadenopathy-associated virus (HTLV-III/LAV/ARV). This virus resembles the lentivirus group and has a propensity to infect stimulated CD4 T cells. It has been isolated from all forms of AIDS and ARC, from healthy individuals with risk factors associated with AIDS, and from saliva, tears, and semen from patients with AIDS. Epidemiologic studies showed that the virus may have originated in Africa. Evidence of the virus in patients with AIDS and related disorders has been reported in Australia, Canada, Britain, Europe, Haiti, and Africa. It is generally agreed that a previously healthy adult with an intact immune system acquires impaired T cell immunity that predisposes the individual to opportunistic infection and cancer. This immunologic attrition probably occurs over a period of 18 months but may take as

long as 5 years. A number of theories have been proposed to explain the occurrence of AIDS in a previously healthy individual.

Epidemiologic studies of AIDS suggest that HTLV-III/LAV/ARV is transmitted in a manner similar to that of hepatitis B virus (HBV). That AIDS is not due to HBV itself is suggested by the absence of AIDS in other populations with a high incidence of HBV infection, the absence of AIDS associated with HBV infection prior to 1979, and the occurrence of AIDS in some individuals who are HBV-negative.

Cytomegalovirus (CMV) has been proposed as a possible cofactor in AIDS. Antibodies to CMV are found in over 95% of patients with AIDS, and CMV can be cultured from the semen of the majority of male homosexual patients with AIDS. Based on longitudinal studies of patients, recurrent or chronic CMV infection is present. DNA hybridization studies have demonstrated cytomegalovirus RNA in Kaposi's sarcoma tissue obtained from AIDS patients. In spite of the close association of CMV and AIDS, many investigators believe that CMV represents an opportunistic infection. Prior to the onset of the AIDS epidemic, acute or chronic CMV infection was not known to result in persistent T cell immunodeficiency.

An increased incidence of antibodies to EBV is found in patients with AIDS. This may reflect their susceptibility to acute and chronic opportunistic infection rather than a primary cause.

The widespread use of drugs in many patients at risk for AIDS has suggested additional cofactors. Amyl nitrite ("poppers") was initially implicated, but subsequent epidemiologic studies have not confirmed a direct association with AIDS. Genetic factors such as histocompatibility antigens have not demonstrated a clear association with AIDS, with the exception of some association between HLA-DR5 and Kaposi's sarcoma.

Epidemiologic studies have uncovered defined risk factors for AIDS that include homosexuality, multiple sexual partners, intravenous drug abuse, hemophilia, and multiple blood transfusions in a susceptible host. Within the homosexual group, individuals with multiple sex partners and those who are the passive partners in rectal intercourse are at greatest risk. Patients with hemophilia who develop AIDS have a history of treatment with factor VIII concentrate. Blood transfusion-related AIDS has been associated with multiple transfusions and the identification of a donor with an AIDS risk factor, although single transfusions in susceptible hosts may also result in AIDS.

It has been recognized that pediatric patients may develop AIDS. Although the existence of **pediatric AIDS (PAIDS)** has been debated, similar epidemiologic, clinical, and laboratory features suggest that AIDS and PAIDS have a similar if not identical cause. Most patients with PAIDS have mothers who are intravenous drug abusers. In these instances, vertical transmission of an infectious agent is suggested. Instances have been reported of infants born to asymptomatic but HTLV-III/LAV/ARV antibody-positive mothers, with transmission to the mother being from a bisexual male. Infants have also been described with PAIDS following multiple blood transfusions where a donor with a specific risk factor has been identified. The occurrence of PAIDS in infants in association with risk factors similar to adult AIDS and the clustering of cases in geographic areas known to be associated with adult AIDS (New York City, New Jersey, Miami, San Francisco, and Los Angeles) provide a close link with AIDS.

Clinical Features

Patients with AIDS usually have a history suggesting multiple opportunistic infections. However, clinical symptoms may vary among the groups at risk. Homosexual patients have a high incidence of syphilis, gonorrhea, and enteritis secondary to *Giardia* or *Entamoeba histolytica*. Severe chronic diarrhea may be due to *Cryptosporidium*. A history of hepatitis may be obtained in homosexual patients or intravenous drug abusers. Infections due to other viral agents include acute and chronic herpes simplex (HSV) and herpes zoster (HZV), CMV, adenovirus, and EBV. Chronic candidal infection of the mucous membranes is frequently present and, when severe, may result in erosive esophagitis. Central nervous system manifestations may be observed secondary to encephalitis or meningitis as a consequence of viral infection, disseminated toxoplasmosis, or cryptococcosis. HTLV-III/LAV/ARV is felt to be a cause of dementia in AIDS. Leukodystrophy has also been observed. Acute or chronic dyspnea is usually a result of infection with *P carinii*. However, other infectious agents may also be isolated, including bacteria, viruses, and fungi. It is important to recognize that some patients infected with *P carinii* may have minimal respiratory symptoms and a normal chest x-ray. An early laboratory abnormality consists of a mild depression of the P and elevation of the P. Diagnosis of *P carinii* infection may require invasive diagnostic techniques including bronchoscopy and lung biopsy. Recent studies suggest that a gallium scan may assist in the diagnosis and follow-up of patients with *P carinii* infection.

Prior to its association with AIDS, Kaposi's sarcoma was found primarily in elderly men of Italian or Jewish origin. Kaposi's sarcoma is of vascular endothelial origin, and an association with CMV has been found. Patients with Kaposi's sarcoma often give a history of fatigue, weight loss, fevers, and night sweats. The typical lesions appear as dark-blue or purple-brown plaques or nodules most commonly on the extremities, but they may appear anywhere on the skin or mucous membranes. Isolated mucosal lesions without skin involvement may be an initial presentation. Biopsy specimens of lesions demonstrate characteristic histologic features including atypical spindle cells, capillarylike spaces, and erythrocytes. Other cancers occur with increased frequency in patients with AIDS, including non-Hodgkin lymphoma, squamous carcinoma of the oral cavity, and cloacogenic carcinoma of the rectum.

In patients with lymphadenopathy syndrome, lymphadenopathy, splenomegaly, fevers, weight loss, and chronic diarrhea are frequent complaints. Lymph nodes may spontaneously increase or decrease in size. Lymph node biopsies usually show reactive hyperplasia, but on occasion malignant cells are observed.

The clinician caring for a patient with AIDS, regardless of the specific form, must be alert to the possibility that one or more infectious agents may account for the symptoms. Agents isolated from patients with AIDS include a variety of viral, bacterial, protozoal, and fungal pathogens (Table 20–10).

Patients with PAIDS are frequently well during the first several months of life. Subsequent clinical features are similar to those reported in patients with adult AIDS and include fevers, failure to thrive, recurrent and chronic pulmonary infection, chronic diarrhea, lymphadenopathy, and hepatosplenomegaly. An eczematous skin eruption is frequently found. Chronic interstitial lung disease of unknown cause may also be present. A feature not found in adult AIDS is the frequent occurrence of parotitis of unknown origin. With one other exception (sexually transmitted disease in AIDS), the acute and chronic infections found in AIDS and PAIDS are similar.

In those instances where an exposure to a specific risk factor can be identified, the incubation period for AIDS is approximately 18 months. The incubation period for PAIDS is shorter, with a mean of 8 months, but may be as long as 5 years. Multiple cases of AIDS have been linked to a single individual. It is generally agreed that AIDS cannot be easily transmitted and that direct inoculation of infected material must occur, eg, intimate sexual contact, sharing of hypodermic needles, blood transfusion, or vertical transmission from mother to infant. The epidemiology of AIDS suggests a transmission similar to that of HBV, and therefore the care and management of a patient with AIDS should be similar. There are no documented cases of transmission of AIDS to health care workers.

Immunologic Diagnosis

The documentation of immunologic abnormalities is essential to establishing a diagnosis of AIDS. Patients with opportunistic infection have the most severe degree of immunodeficiency. Lymphopenia is marked ($< 600/\mu L$) and is associated with a substantial reduction in the percentage of T cells ($< 30\%$; normal, $> 60\%$). The helper/suppressor T cell ratio (see Chapter 18) is usually less than 0.5 (normal, > 1.7). Functional studies of peripheral blood lymphocytes are abnormal, with a reduced response to the mitogens phytohemagglutinin and pokeweed and a reduced to absent response to antigens. Evaluation of polyclonal B cell immunity demonstrates elevated levels of all immunoglobulin classes. Although antibody to a variety of infectious agents may be demonstrated, patients usually fail to respond with appropriate specific antibody production following immunization. Most patients have circulating immune complexes. NK cell activity and specific cytotoxicity against virally infected cells is reduced. Lymphokine production may be abnormal, with diminished interleukin-2 production by T cells and increased production of an acid-labile alpha interferon.

Table 20–10. Principal agents of infection in patients with acquired immunodeficiency syndrome (AIDS).

Viruses	**Mycobacteria**
Herpesvirus (types 1 and 2)	*Mycobacterium tuberculosis*
Cytomegalovirus	*Mycobacterium avium-intracellulare*
Varicella	*Mycobacterium kansasii*
Adenovirus	*Legionella* sp
Epstein-Barr	**Spirochetes**
Retrovirus (HTLV-I, -III)	*Treponema* sp (including *Treponema pallidum*)
Fungi	**Bacteria**
Candida albicans	*Campylobacter* sp
Cryptococcus neoformans	*Neisseria* sp (including *Neisseria gonorrhoeae*)
Nocardia	*Shigella* sp
Protozoa	*Salmonella* sp
Pneumocystis carinii	*Chlamydia*
Toxoplasma gondii	
Isospora sp	
Cryptosporidium	
Giardia lamblia	
Entamoeba histolytica	

Patients with Kaposi's sarcoma also have significantly impaired T and B cell immunity, but it is less severe in this group than that found in patients with opportunistic infection. Other abnormalities, eg, decreased NK cell activity and increased acid-labile interferon, are also found in this group of patients. As expected, immunosuppressive therapy for the treatment of Kaposi's sarcoma may result in additional impairment of immunity.

Patients with the lymphadenopathy syndrome have mild to moderate impairment of T cell immunity. Immunoglobulin levels are usually elevated. Although the helper/suppressor T cell ratio is reversed, values in this group fall between 0.5 and low normal.

Healthy homosexual males have been shown to have reduced helper/suppressor T cell ratios. Unlike the ratio in patients with Kaposi's sarcoma or opportunistic infection, the reversed ratio in these individuals is usually the result of an increased percentage of suppressor cells with a normal percentage of helper cells. Abnormal T and B cell function has not been demonstrated in healthy homosexuals. The significance of the reversed helper/suppressor T cell ratio in this group of individuals is not known. Long-term follow-up studies will be required to determine if they are transient abnormalities or are predictive of progressive disease. One should use caution in diagnosing AIDS based on a reversed helper/suppressor T cell ratio alone, since this abnormality may also be found following acute viral infection.

Complement levels are normal or increased in AIDS. Most patients have circulating immune complexes. Phagocytosis is normal. Monocytes may not function normally in T cell cooperation. Increased levels of thymosin alpha-1, a putative thymic hormone,

have been described. β_2-Microglobulin may also be elevated in serum samples.

Patients with PAIDS and hemophilia patients with AIDS have immunologic abnormalities similar to those described for patients with Kaposi's sarcoma or opportunistic infection.

Differential Diagnosis

A diagnosis of AIDS can be established by utilizing combined epidemiologic, clinical, and laboratory data. Although the diagnosis of AIDS cannot be made in an individual who is antibody-negative for HTLV-III/LAV/ARV, the presence of antibody does not establish a diagnosis, since there are many healthy individuals who are positive for antibody. Difficulty in diagnosis may be encountered in young patients (infants and children), older males, individuals who lack the complete syndrome, and individuals in whom a risk factor cannot be identified. The older male patient with traditional Kaposi's sarcoma who has not received immunosuppressive therapy will usually have normal immunologic findings. Acquired hypogammaglobulinemia may be associated with T cell immunodeficiency, but this disorder may be clearly distinguished from AIDS by the absence of immunoglobulins in the former. Patients with cancer who receive immunosuppressive therapy frequently develop opportunistic infection. A careful history to elicit risk factors associated with AIDS may be required, especially if the cancer is Kaposi's sarcoma, lymphoma, or lymphosarcoma. The lymphadenopathy syndrome may be difficult to differentiate from cancer. PAIDS in infants should be differentiated from congenital or inherited forms of immunodeficiency. Specifically, severe combined immunodeficiency, Wiskott-Aldrich syndrome, and combined immunodeficiency with enzyme deficiency should be ruled out by laboratory evaluation. (See discussions of these disorders, above.) The diagnosis in infants under 6 months of age should be made only by viral isolation, since antibody may be transmitted from the mother.

Treatment

No treatment capable of reversing the immunodeficiency of AIDS is available. Aggressive diagnostic measures are required to identify microbial agents that might require specific therapy. Antiviral agents such as acyclovir may be successful in the treatment of herpes simplex and herpes zoster. Mycobacterial infection usually requires therapy with multiple agents, but the therapy is relatively ineffective. *P carinii* infection does not respond readily to treatment with pentamidine or trimethoprim-sulfamethoxazole. It is not clear at present whether these agents should be used separately or concomitantly. Reactions to trimethoprim-sulfamethoxazole are observed in 30–40% of patients and consist of skin rash, fever, hepatosplenomegaly, and thrombocytopenia. Amphotericin B is required for the treatment of systemic candidal infection. Antibiotic therapy for other infections should be carefully selected for current sensitivities. No antimicrobial therapy is available for many infections, eg, with *Cryptosporidium*, EBV. Experimental agents are being evaluated to treat HTLV-III/LAV/ARV, eg, suramin, ribavirin, azidothymidine, and HPA-23, all of which are inhibitors of reverse transcriptase. DHPG (dihydropropoxymethoxyguanine) has been used to treat CMV infection.

Debate exists about the most effective treatment for Kaposi's sarcoma. In many treatment series, the number of deaths due to infection is greater than that due to cancer. Because many of the treatment protocols utilize immunosuppressive agents such as radiation therapy or cytotoxic agents, increased immunodeficiency usually results. Radiation therapy is recommended for limited disease. Single-agent therapy is recommended for slowly progressive disease, and combination drug treatment (doxorubicin, bleomycin, vinblastine) is used for patients with rapidly progressive disease. Trials of immunomodulating agents such as alpha interferon, gamma interferon, and interleukin, directed at enhancing immunologic function, are under investigation.

Attempts at immunologic reconstitution have not been successful in patients with AIDS. Thymic factor, interferon, and bone marrow transplantation have been attempted without long-term improvement in immunologic function. Because of the severe degree of T cell deficiency present in patients with AIDS, blood products given to these patients should be radiated to prevent GVH reaction. Prophylactic trimethoprim-sulfamethoxazole should be given when tolerated.

Although there is no evidence of transmission of AIDS to hospital patients or personnel, precautions should be taken to prevent the inadvertent inoculation of AIDS material. Patients with AIDS or individuals with a risk factor associated with AIDS should not be blood donors. Blood banks and blood product industries must use HTLV-III/LAV/ARV screening of donors to prevent transmission of AIDS by blood products.

Complications & Prognosis

All patients with opportunistic infection and half of patients with Kaposi's sarcoma have died after 2 years of follow-up. Patients with lymphadenopathy syndrome have a better prognosis. Patients with PAIDS have a mortality rate similar to that of AIDS. The long-term outcome of HTLV-III/LAV/ARV antibody-positive, asymptomatic individuals has not been determined.

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21 Rheumatic Diseases

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Many of the major rheumatologic disorders are autoimmune in nature. Therefore, a thorough understanding of the mechanisms of the immune response is essential to the rheumatologist. In this chapter we shall discuss the rheumatologic diseases with proved or hypothesized immunologic pathogenesis. It might be helpful to refer to the previous chapter on autoimmunity if questions arise during the study of the rheumatic diseases.

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Major Immunologic Features

- High-titer antinuclear antibodies (diffuse or outline pattern on immunofluorescence).
- Anti-single-stranded and anti-double-stranded DNA and anti-Sm antibodies.
- Depressed serum complement levels.
- Deposition of immunoglobulin and complement along glomerular basement membrane and at the dermal-epidermal junction.
- Numerous other autoantibodies.

General Considerations

Osler first described the systemic manifestations of systemic lupus erythematosus (SLE) in 1895. Prior to that time, lupus was considered to be a disfiguring but nonfatal skin disease. It is now known to be a chronic systemic inflammatory disease that follows a course of alternating exacerbations and remissions. Multiple organ system involvement characteristically occurs during periods of disease activity. The cause is not known. The disease affects predominantly females (4:1 over males) of childbearing age; however, the age at onset ranges from 2 to 90 years. The incidence is higher among nonwhites (particularly blacks) than whites.

Immunologic Pathogenesis

The discovery of the lupus erythematosus (LE) cell phenomenon (see Immunologic Diagnosis, below) marked the start of the modern era of research into the pathogenesis of systemic lupus erythematosus. This initial clinical observation led to the finding of antinuclear factors and antibodies to DNA in the sera of patients with SLE. Further studies of renal eluates from patients with SLE established the importance of DNA-containing immune complexes in the causation of lupus glomerulonephritis. Reduced serum complement and the presence of antibodies to double-stranded (ds) DNA have become routine correlates of active SLE, distinguishing this entity from other lupus variants. The cause of antibody formation to DNA is uncertain. Antibodies to ds-DNA cannot be provoked by experimental immunization and occur almost exclusively in SLE and in a number of mouse models for SLE, such as MRL/1 and New Zealand black (NZB) mice. It is not known whether viral or host DNA is the immunogen for anti-DNA antibody formation.

Additional autoantibody activity is also associated with the pathogenesis of SLE. Lymphocytotoxic antibodies (with predominant specificity for T lymphocytes) occur in many patients with SLE and in NZB mice. Such antibodies are capable of killing T lymphocytes in the presence of complement and of coating peripheral blood T cells so as to interfere with HLA typing and with certain functional activities such as the proliferative response to alloantigens. These antibodies have specificity for T cell surface antigens and can be released from the lymphocyte cell surface in the form of specific antigen-antibody complexes. Such complexes may themselves attach to and block the function of other lymphocytes or may contribute to immune complex deposition, leading to vasculitis and nephritis. Autoantibody formation is in part genetically determined; eg, patients with HLA-DR2 are more likely to produce anti-ds-DNA antibodies, whereas those with HLA-DR3 and HLA-DR7 produce anti-SS-A antibodies.

Autoantibody formation is normally prevented through the action of T regulatory lymphocytes called suppressor T cells. Although the mechanism of suppression is unknown, such suppressor T cells probably play an important role in immunologic tolerance and self/nonself discrimination (see Chapter 11). A deficiency of suppressor T cells has been demonstrated in NZB mice. A defect in suppressor T cell activity has also been observed in human beings with SLE; however, this defect may be due to anti-T cell antibody activity and may not represent a primary suppressor T cell deficiency.

A number of human family studies have demonstrated a genetic susceptibility to the development of SLE. It has now been shown that lupus patients share common B cell alloantigens. What role these alloantigens play in the pathogenesis of SLE has yet to be de-

termined. Furthermore, there appears to be an inherited deficiency in cell surface C3b receptors in patients with SLE. This defect may impair the ability to clear circulating immune complexes, thereby amplifying the autoimmune process.

SLE, like many rheumatic disorders, occurs predominantly in women. Studies have demonstrated that estrogens enhance anti-DNA antibody formation and increase the severity of renal disease in laboratory models. Androgens have an opposite effect on both anti-DNA antibody production and renal disease.

Clinical Features

A. Symptoms and Signs: SLE presents no single characteristic clinical pattern. The onset can be acute or insidious. Constitutional symptoms include fever, weight loss, malaise, and lethargy. Every organ system may become involved.

1. Skin—The most common skin lesion is an erythematous rash involving areas of the body chronically exposed to ultraviolet light. Relatively few patients with SLE develop the classic "butterfly" rash or the characteristic erythematous rash over the fingertips and palms. In some cases, the rash is similar in appearance to that of discoid lupus erythematosus. The rash may resolve without sequelae or may result in scar formation, atrophy, and hypo- or hyperpigmentation. In addition, bullae, patches of purpura, urticaria, angioneurotic edema, patches of vitiligo, subcutaneous nodules, and thickening of the skin may be seen. Vasculitic lesions, ranging from palpable purpura to digital infarction, are common. Alopecia, which may be diffuse, patchy, or circumscribed, is also common. Mucosal ulcerations, involving both oral and genital mucosa, are present in about 15% of cases. Raynaud's phenomenon occurs in about 15% of patients with SLE.

2. Joints and muscles—Polyarthralgia or arthritis is the most common manifestation of SLE (90%). The arthritis is symmetric and can involve almost any joint. It may resemble rheumatoid arthritis, but bony erosions and severe deformity are unusual.

Tenosynovitis seldom occurs. Avascular necrosis of bone is a frequent occurrence in SLE. The femoral head is most frequently affected, but other bones may also be involved. Corticosteroids, which are major therapeutic agents in SLE, may play a role in the pathogenesis of this complication. Myalgias, with or without frank myositis, are common.

3. Polyserositis—Pleurisy is frequently present. Although one-third of cases have pleural fluid, massive effusion is rare. Involvement of the pleura produces pleuritic chest pain and shortness of breath. Pericarditis is the commonest form of cardiac involvement and can be the first manifestation of SLE. The pericarditis is usually benign, with only mild chest discomfort and a pericardial friction rub, but severe pericarditis leading to tamponade can occur. Peritonitis alone is extremely rare, although 5–10% of patients with pleuritis and pericarditis have concomitant peritonitis. Manifestations of peritonitis include abdominal pain, anorexia, nausea and vomiting, and, rarely, ascites.

4. Lungs—Clinically apparent lupus pneumonitis is unusual. When a pulmonary infiltrate develops in a patient with SLE, particularly one being treated with corticosteroids or immunosuppressive drugs, infection must be the first diagnostic consideration. The commonest form of lupus pulmonary involvement is restrictive interstitial lung disease, which may be asymptomatic and detectable only by pulmonary function tests. The chest x-ray is usually normal but may show "platelike" atelectasis or interstitial fibrosis with "honey-combing." Other pulmonary manifestations include pulmonary hypertension, alveolar hemorrhage, pneumothorax, hemothorax, and vasculitis.

5. Heart—Clinically apparent myocarditis occurs rarely in SLE but when present may result in congestive heart failure with tachycardia, gallop rhythm, and cardiomegaly. Arrhythmias are unusual and are considered a preterminal event. The endocarditis of SLE is very difficult to diagnose. The verrucous endocarditis of SLE, with the characteristic Libman-Sacks vegetations, is usually diagnosed only at autopsy. Thickening of the aortic valve cusps with resultant aortic insufficiency can occur. Coronary artery disease, possibly related to corticosteroid therapy, is being detected with increasing frequency.

6. Kidney—Renal involvement is a frequent and serious feature of SLE. Seventy-five percent of patients have nephritis at autopsy. The study of renal tissue by light microscopy, immunofluorescence, and electron microscopy has revealed 4 histologic lesions associated with rather distinctive clinical features. (1) Mesangial glomerulonephritis is characterized by hypercellularity and the deposition of immune complexes in the mesangium. This is a benign form of lupus nephritis. (2) In focal glomerulonephritis, segmental proliferation occurs in less than 50% of glomeruli. Immune complexes are deposited in the mesangium and in the subendothelium of the glomerular capillary. Focal glomerulonephritis is usually a benign process, but on occasion it may progress to a diffuse proliferative lesion. (3) Diffuse proliferative glomerulonephritis is characterized by extensive cellular proliferation in more than 50% of glomeruli. Immune complexes are deposited largely in subendothelial distribution. This process frequently leads to renal failure. (4) In membranous glomerulonephritis, glomerular cellularity is normal, but the capillary basement membrane is thickened. Immune complexes are deposited mainly in subepithelial and intramembranous areas. This lesion may be associated with the development of renal failure.

It must be emphasized that in individual patients, a benign renal lesion may evolve into a more serious one.

Systemic hypertension is a common finding in acute or chronic lupus nephritis and may contribute to renal dysfunction.

7. Nervous system—Cerebral involvement is a life-threatening complication of SLE. Disturbances of

mentation and aberrant behavior, such as psychosis or depression, are the commonest manifestations of central nervous system involvement. Convulsions, cranial nerve palsies, aseptic meningitis, migraine headache, peripheral neuritis, and cerebrovascular accidents may also occur.

8. Eye—Ocular involvement is present in 20–25% of patients. The characteristic retinal finding (the cytoid body) is a fluffy white exudative lesion caused by focal degeneration of the nerve fiber layer of the retina secondary to retinal vasculitis. Scleritis is also a manifestation of ocular vasculitis. Corneal ulceration occurs in conjunction with Sjögren's syndrome (see below).

9. Gastrointestinal system—Gastrointestinal ulceration due to vasculitis can occur in SLE but is uncommon. Pancreatitis is not unusual, and acute and chronic hepatitis may occur.

10. Hematopoietic system—See Laboratory Findings, below.

11. Vascular system—Small-vessel vasculitis commonly occurs in active SLE. Cutaneous manifestations of small-vessel disease include splinter hemorrhages, periungual occlusions, finger pulp infarctions, and atrophic ulcers. Gastrointestinal manifestations include abdominal pain, diarrhea, hemorrhage, pancreatitis, and cholecystitis. The "stocking-glove" peripheral neuropathy commonly encountered in SLE is due to small-vessel vasculitis. Medium-vessel arteritis, involving small arteries 0.5–1 mm in diameter, also occurs in SLE. Manifestations range from bowel infarction to mononeuritis multiplex to cerebrovascular accidents.

12. Sjögren's syndrome—Five to 10% of patients with SLE develop the sicca complex (keratoconjunctivitis sicca, xerostomia).

13. Drug-induced lupuslike syndrome—Certain drugs may provoke a lupuslike picture in susceptible individuals. The most commonly implicated drugs, hydralazine and procainamide, can induce arthralgias, arthritis, skin rash, and, less commonly, fever and pleurisy. Nephritis and central nervous system involvement are thought not to occur. The serologic picture is similar to that of SLE, but antibody to native ds-DNA occurs only rarely. The disease usually remits when the drug is discontinued. The list of agents that produce a lupuslike syndrome is increasing rapidly and includes phenytoin, trimethadione, mephenytoin, isoniazid, aminosalicylic acid, penicillin, tetracyclines, penicillamine, sulfonamides, streptomycin, griseofulvin, phenylbutazone, oral contraceptives, methyl- and propylthiouracil, methyldopa, levodopa, pindolol, aminoglutethimide, and acebutolol.

B. Laboratory Findings: Anemia is the most common hematologic finding in SLE. Eighty percent of patients present with a normochromic, normocytic anemia due to marrow suppression. A few develop Coombs-positive hemolytic anemia. Leukopenia and thrombocytopenia occur commonly. Urinalysis may show hematuria, proteinuria, and red and white cell casts. The sedimentation rate is high in almost all cases. Serologic abnormalities are described in the section on immunologic diagnosis (below). The synovial fluid in SLE is yellow and clear, with a low viscosity. The white cell count does not exceed 4000/μL, most of which are lymphocytes. Complement levels are low. The pleural effusion of SLE is a transudate with a predominance of lymphocytes and a total white cell count of no more than 3000/μL. A hemorrhagic pleural effusion is very rare. In central nervous system lupus, the cerebrospinal fluid protein concentration is sometimes elevated, and there is occasionally a mild lymphocytosis. Patients with nonfocal central nervous system disease may have antineuronal antibodies in cerebrospinal fluid.

There are numerous characteristic pathologic changes in SLE:

(1) The verrucous endocarditis of Libman-Sacks consists of ovoid vegetations, 1–4 mm in diameter, which form along the base of the valve and, rarely, on the chordae tendineae and papillary muscles.

(2) A peculiar periarterial concentric fibrosis results in the so-called "onion skin" lesion seen in the spleen.

(3) The pathognomonic finding in SLE, the "hematoxylin body," consists of a homogeneous globular mass of nuclear material that stains bluish purple with hematoxylin. Hematoxylin bodies have been found in the heart, kidneys, lungs, spleen, lymph nodes, and serous and synovial membranes. It should be emphasized that patients with fulminant SLE involving the central nervous system, skin, muscles, joints, and kidneys may not have any distinctive pathologic abnormalities at autopsy.

C. X-Ray and Other Findings: Chest x-ray may reveal cardiomegaly (due either to pericarditis or myocarditis), pleural effusion, platelike atelectasis, or interstitial fibrosis with a "honeycomb" appearance. Joint x-rays may show soft tissue swelling and mild osteopenia but rarely show erosions. The lumbar puncture, cerebrospinal fluid, EEG, and radionuclide brain scan are abnormal in many cases of central nervous system involvement.

Immunologic Diagnosis

A. Proteins and Complement: Most patients with SLE (80%) present with elevated α_2- and γ-globulins. Hypoalbuminemia is occasionally present. The serum complement is frequently reduced in the presence of active disease because of increased utilization due to immune complex formation, reduced synthesis, or a combination of both factors. Several complement components, including C3 and C4, and total hemolytic complement activity are decreased while the activity of the attack complex of complement, C5–9, is increased during disease activity. The serum of patients with active SLE occasionally contains circulating cryoglobulin complexes of IgM/IgG aggregates and complement that will precipitate in the cold.

B. Autoantibodies:

1. LE cell phenomenon—This phenomenon was first described in the bone marrow of patients with

SLE. It reflects the presence of 7S IgG antibody to deoxyribonucleoprotein. However, this relatively cumbersome and insensitive technique is largely of historic interest.

2. Antinuclear antibodies (ANA)—Immunoglobulins of all classes may form antinuclear antibodies. The indirect immunofluorescence technique was introduced in 1957. Six different morphologic patterns of immunofluorescent staining have been described, 4 of which have clinical significance (Fig 21–1 and Table 21–1).

a. The "homogeneous" ("diffuse" or "solid") pattern is the morphologic expression of antihistone antibodies and occurs in patients with systemic or drug-induced lupus erythmatosus. In this pattern, the nucleus shows diffuse and uniform staining.

b. The "peripheral" ("shaggy" or "outline") pattern is the morphologic expression of anti-ds-DNA antibodies. The outline pattern is best seen when human leukocytes are used as substrate. It is characteristic of active SLE.

c. The "speckled" pattern reflects the presence of antibodies directed against non-DNA nuclear constituents. The antigens to which these antibodies are directed can be extracted from the nucleus using saline. The anti-ENA (extractable nuclear antigen) assay detects antibodies against 2 extractable nuclear antigens, the Sm (Smith) antigen and RNP (ribonucleoprotein) antigen. Antibodies against the Sm antigen are characteristic of SLE. High titers of anti-RNP antibodies are the hallmark of mixed connective tissue disease, but low-titer anti-RNP antibodies may occur in SLE. Other antinuclear antibodies have been described in Sjögren's syndrome, rheumatoid arthritis, scleroderma, and polymyositis-dermatomyositis.

d. The "nucleolar" pattern is caused by the homogeneous staining of the nucleolus. It has been suggested that this antigen may be the ribosomal precursor of ribonucleoprotein. This pattern is most often associated with scleroderma or polymyositis-dermatomyositis.

All the nuclear staining patterns must be interpreted with caution for the following reasons: (1) The serum of a patient with any rheumatic disease may contain many autoantibodies to different nuclear constituents, so that a "homogeneous" pattern may obscure a "speckled" or "nucleolar" pattern; (2) different antibodies in the serum can be present in different titers, so that by diluting the serum one can change the pattern observed; (3) the stability of the different antigens is different and can be changed by fixation or denaturation; and (4) the pattern observed appears to be influenced by the types of tissues or cells used as substrate for the test.

The ANA determination is occasionally positive in normal individuals, in patients with various chronic diseases, and in the aged. However, high titers are most often associated with SLE. Absence of ANA is strong evidence against a diagnosis of SLE.

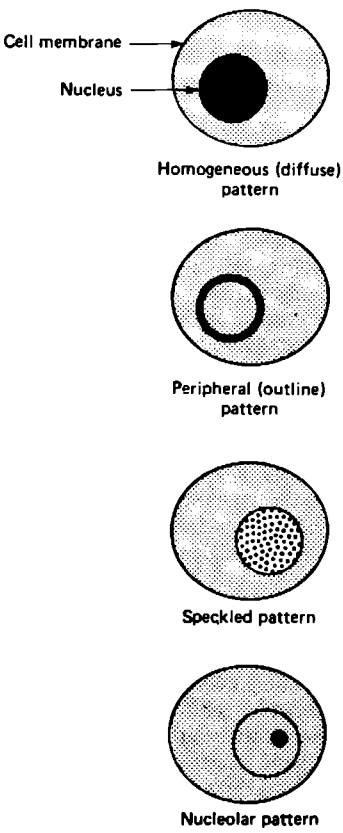

Figure 21–1. Patterns of immunofluorescent staining for antinuclear antibodies.

Table 21–1. Antinuclear antibodies.

Pattern	Antigen	Associated Diseases
Peripheral	Double-stranded DNA	SLE
Homogeneous	DNA-histone complex	SLE, occasionally other connective tissue disease
Speckled	Sm (Smith antigen)	SLE
	RNP (ribonucleoprotein)	Mixed connective tissue disease, SLE, Sjögren's syndrome, scleroderma, polymyositis
	SS-A (Ro)	Sjögren's syndrome, SLE
	SS-B (La)	Sjögren's syndrome, SLE
	Jo-1	Polydermatomyositis
	PM-Sc1	Polymyositis, scleroderma
	Centromere	CREST syndrome
	RANA (rheumatoid-associated nuclear antigen) (nuclear antigen induced by EB virus)	Rheumatoid arthritis
Nucleolar	Nucleolus-specific RNA	Scleroderma

3. Anti-DNA antibodies–Three major types of anti-DNA antibodies can be found in the sera of lupus patients: (1) anti-single-stranded or "denatured" DNA (ss-DNA); (2) anti-double-stranded or "native" DNA (ds-DNA); and (3) antibodies that react to both ss-DNA and ds-DNA. These antibodies may be either IgG or IgM immunoglobulins. High titers of anti-ds-DNA antibodies are essentially seen only in SLE. In contrast, anti-ss-DNA antibodies are not specific and can be found in other autoimmune diseases, eg, rheumatoid arthritis, chronic active hepatitis, and primary biliary cirrhosis. Furthermore, antibodies to ss-DNA occur in drug-induced lupuslike syndrome and can be induced in experimental animals by the injection of DNA complexed to protein and emulsified in Freund's complete adjuvant. Antibodies to DNA can be quantitatively measured by a radioimmunoassay using labeled DNA. Complement-fixing and high-avidity anti-ds-DNA antibodies may be associated with the development of renal disease. The amount of antibody correlates well with disease activity, and the antibody titer frequently decreases when patients enter remission. Circulating immune complexes are present in the sera of patients with active disease. However, different assay techniques are required to detect complexes of different sizes, and there is controversy about how closely the level of soluble circulating immune complexes correlates with disease activity.

4. Antierythrocyte antibodies–These antibodies belong to all major immunoglobulin classes and can be detected by the direct Coombs test. The prevalence of these antibodies among SLE patients ranges from 10% to 65%. Hemolytic anemia does occasionally occur and, when present, is associated with a complement-fixing warm anticrythrocyte antibody.

5. Circulating anticoagulants and antiplatelet antibodies–A circulating anticoagulant that prolongs the partial thromboplastin and prothrombin times develops in 10–15% of patients with SLE. It appears to be an antibody directed against phospholipid, and for this reason it occurs with increased frequency in patients with a false-positive VDRL. Interestingly, the lupus anticoagulant has anti-ds-DNA activity. Hemorrhagic complications are rare, but paradoxic thrombotic states may develop, possibly from inhibition of prostacyclin formation or from low levels of antithrombin III. Specific anti-factor VIII antibodies have also been described. These antibodies are potent anticoagulants and may be associated with bleeding. Antiplatelet antibodies are found in 75–80% of patients with SLE. These antibodies inhibit neither clot retraction nor thromboplastin generation in normal blood. They probably induce thrombocytopenia by direct effects on platelet surface membrane.

6. False-positive serologic test for syphilis–A false-positive VDRL test is seen in 10–20% of patients with SLE. The serologic test for syphilis can be considered an autoimmune reaction, because the antigen is a phospholipid present in many human organs (see above).

7. Rheumatoid factors–Almost 30% of patients with SLE have a positive latex fixation test for rheumatoid factors.

8. Anticytoplasmic antibodies–Numerous anticytoplasmic antibodies (antimitochondrial, antiribosomal, antilysosomal) have been found in patients with SLE. These antibodies are not organ- or species-specific. Antiribosomal antibodies are found in the sera of 25–50% of patients. The major antigenic determinant is ribosomal RNA. Antimitochondrial antibodies are more common in other diseases (eg, primary biliary cirrhosis) than in SLE.

C. Tissue Immunofluorescence Studies:

1. Kidney–(See Chapter 28.) Irregular or granular accumulation of immunoglobulin and complement occurs along the glomerular basement membrane and in the mesangium in patients with lupus nephritis. On electron microscopy, these deposits are seen in subepithelial, subendothelial, and mesangial sites.

2. Skin–Almost 90% of patients with SLE have immunoglobulin and complement deposition in the dermal-epidermal junction of skin that is *not* involved with an active lupus rash. The immunoglobulins are IgG or IgM and appear as a brightly staining homogeneous or granular band. Patients with discoid lupus erythematosus show deposition of immunoglobulin and complement only in involved skin (see Chapter 29).

Differential Diagnosis

The diagnosis of SLE in patients with classic multisystem involvement and a positive ANA test is not difficult. However, the onset of the disease can be vague and insidious and can therefore present a perplexing diagnostic problem. The polyarthritis of SLE is often similar to that seen in viral infections, infective endocarditis, mixed connective tissue disease, rheumatoid arthritis, and rheumatic fever. When Raynaud's phenomenon is the predominant complaint, progressive systemic sclerosis should be considered. SLE can present with a myositis similar to that of polymyositis-dermatomyositis. The clinical constellation of arthritis, alopecia, and a positive VDRL suggests secondary syphilis. Felty's syndrome (thrombocytopenia, leukopenia, splenomegaly in patients with rheumatoid arthritis) can simulate SLE. Takayasu's disease should be considered in a young woman who presents with arthralgias, fever, and asymmetric pulses.

Sometimes the diagnosis of SLE can be facilitated by finding anti-ds-DNA or a high titer of ANA (outline pattern) in serum. Some patients with discoid lupus erythematosus may develop leukopenia, thrombocytopenia, hypergammaglobulinemia, a positive ANA, and an elevated sedimentation rate. Ten percent of patients with discoid lupus erythematosus have LE cells and associated mild systemic symptoms. Although the exact relationship between SLE and discoid lupus erythematosus is uncertain, the frequent presence of anti-ds-RNA in discoid lupus erythematosus suggests that they are parts of a single disease spectrum.

Treatment

The efficacy of the drugs used in the treatment of SLE is difficult to evaluate, since spontaneous remissions do occur. There are few controlled studies, because it is difficult to withhold therapy in the face of the life-threatening disease that can develop in fulminant SLE. Depending on the severity of the disease, no treatment, minimal treatment (aspirin, antimalarials), or intensive treatment (corticosteroids, cytotoxic drugs) may be required.

When arthritis is the predominant symptom and other organ systems are not significantly involved, high-dose aspirin or another fast-acting nonsteroidal anti-inflammatory drug may suffice to relieve symptoms. When the skin or mucosa is predominantly involved, antimalarials (hydroxychloroquine or chloroquine) and topical corticosteroids are very beneficial. Because high-dosage antimalarial therapy may be associated with irreversible retinal toxicity, these drugs should be used judiciously and in low doses.

Systemic corticosteroids in severe SLE can suppress disease activity and prolong life. The mode of action is unknown, but the immunosuppressive and anti-inflammatory properties of these agents presumably play a significant role in their therapeutic efficacy. High-dosage corticosteroid treatment (eg, prednisone, 1 mg/kg/d orally) decreases γ-globulin levels and autoantibody titers and suppresses immune responses. High-dosage corticosteroid therapy is recommended in acute fulminant lupus, acute lupus nephritis, acute central nervous system lupus, acute autoimmune hemolytic anemia, and thrombocytopenic purpura. Recent work suggests that one or more courses of "pulse" therapy (ie, 15 mg/kg/d intravenously for 3 days) may be effective in patients with recalcitrant disease. The course of corticosteroid therapy should be monitored by the clinical response and meticulous follow-up of laboratory and immunologic parameters—complete blood count with reticulocyte and platelet counts, urinalysis, anti-ds-DNA titer, and complement levels.

If the clinical and immunologic status of the patient fails to improve or if serious side effects of corticosteroid therapy develop, immunosuppressive therapy with cytotoxic agents such as cyclophosphamide, chlorambucil, or azathioprine is indicated. Because of serious complications (cancer, marrow suppression, infection, and liver and gastrointestinal toxicity), immunosuppressive agents should be used with discretion.

Complications & Prognosis

SLE may run a very mild course confined to one or a few organs, or it may be a fulminant fatal disease. Renal failure and central nervous system lupus were the leading causes of death until the corticosteroids and cytotoxic agents came into widespread use. Since then, the complications of therapy, including atherosclerosis, infection, and cancer, have become common causes of death. The 5-year survival rate of patients with SLE has markedly improved over the past decade and now approaches 80–90%.

RHEUMATOID ARTHRITIS

Major Immunologic Features

- 7S and 19S IgM and 7S IgG rheumatoid factors in serum and synovial fluid.
- Decreased complement in synovial fluid.

General Considerations

Rheumatoid arthritis is a chronic recurrent, systemic inflammatory disease primarily involving the joints. Constitutional symptoms include malaise, fever, and weight loss. The disease characteristically begins in the small joints of the hands and feet and progresses in a centripetal and symmetric fashion. Deformities are common. Extra-articular manifestations are characteristic of the rheumatoid process and often cause significant morbidity. Extra-articular manifestations include vasculitis, atrophy of the skin and muscle, subcutaneous nodules, lymphadenopathy, splenomegaly, and leukopenia.

The disease affects 1–3% of Americans, with a female to male ratio of 3:1.

Immunologic Pathogenesis

The antigenic stimulus that initiates the immune response and subsequent inflammation in rheumatoid arthritis is unknown. An increased prevalence of HLA-D4 and HLA-DR4 occurs in patients with rheumatoid arthritis. It is possible that these and perhaps other genetic determinants impart a genetic susceptibility to an unidentified environmental factor, such as a virus, that initiates the disease process. Although no virus particles have ever been identified, it is likely that an antigenic stimulus leads to the appearance of an abnormal IgG that results in the production of rheumatoid factor and the eventual development of rheumatoid disease (Fig 21–2).

Recent studies have suggested a possible relationship between EB virus and rheumatoid arthritis. Rheumatoid patients have a high frequency of precipitating serum antibody (RA precipitin, RAP) that reacts specifically with a nuclear antigen from a human lymphoblastoid cell line containing EB virus. This antigen (RA nuclear antigen, RANA) is only expressed in EB virus-infected cells. The EB virus is a polyclonal stimulator of B cells and can lead to the in vitro production of rheumatoid factor by human B cells. However, because of the high frequency of RAP in normal controls, any causal relationship of EB virus with rheumatoid arthritis remains speculative.

Synovial lymphocytes produce IgG that is recognized as foreign and stimulates an immune response within the joint, with production of 7S IgG, 7S IgM, and 19S IgM anti-immunoglobulins, ie, rheumatoid factors. The presence of IgG aggregates or IgG-rheumatoid factor complexes results in activation of

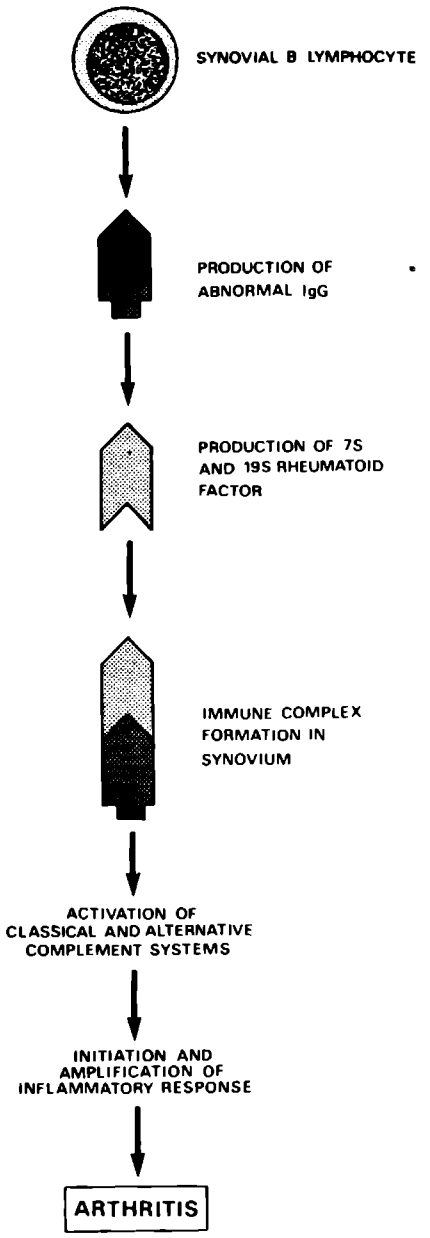

Figure 21–2. Hypothetical immunopathogenesis in rheumatoid arthritis.

the classic complement system. Breakdown products of complement accumulate within the joint and amplify the activation of complement by stimulation of the alternative (properdin) system. Activation of the complement system results in a number of inflammatory phenomena, including histamine release, the production of factors chemotactic for PMNs and mononuclear cells, and membrane damage with cell lysis (see Chapter 10). There is a marked influx of white cells into the synovial space. Prostaglandins and leukotrienes produced by inflammatory cells are thought to play a major role in mediation of the inflammatory process. In addition, activated lysosomes and enzymes released into the synovial space by leukocytes further amplify the inflammatory and proliferative response of the synovium. The mononuclear infiltrate characteristically seen within the synovium includes perivascular collections of helper T cells and interstitial collections of suppressor T cells. B lymphocytes, lymphoblasts, plasma cells, and macrophages. The immunologic interaction of these cells leads to the liberation of lymphokines responsible for the accumulation of macrophages within the inflammatory synovium and to continued immunoglobulin and rheumatoid factor synthesis. Immune complexes in articular cartilage attract PMNs, which damage cartilage by releasing proteases and collagenase.

Rheumatoid factor may play a role in the causation of extra-articular disease. Patients with rheumatoid vasculitis have high titers of 19S and 7S IgM and 7S IgG rheumatoid factors. Antigen-antibody complexes infused into experimental animals in the presence of IgM rheumatoid factor induce necrotizing vasculitis. Theoretically, immune complexes initiate vascular inflammation by the activation of complement. Pulmonary involvement is associated with the deposition of 11S and 15S protein complexes containing IgG in the walls of pulmonary vessels and alveoli. 19S IgM rheumatoid factor has also been detected in arterioles and alveolar walls adjacent to cavitary nodules. Rheumatoid factors do not initiate the inflammatory process that causes rheumatoid disease, but they probably perpetuate and amplify that process.

Clinical Features

A. Symptoms and Signs:

1. Onset–The usual age at onset is 20–40 years. In most cases the disease presents with joint manifestations; however, some patients first develop extra-articular manifestations, including fatigue, weakness, weight loss, mild fever, and anorexia.

2. Articular manifestations–Patients experience stiffness and joint pain which are generally worse in the morning and improve throughout the day. These symptoms are accompanied by signs of articular inflammation, including swelling, warmth, erythema, and tenderness on palpation. The arthritis is symmetric, involving the small joints of the hands and feet, ie, the proximal interphalangeals, metacarpophalangeals, the wrists, and the subtalars. Large joints (knees, hips, elbows, ankles, shoulders) commonly become involved later in the course of the disease. Although the cervical spine may be involved, the thoracic and lumbosacral spine is usually spared.

Periarticular inflammation is common, with tendonitis and tenosynovitis resulting in weakening of tendons, ligaments, and supporting structures. Joint pain leads to muscle spasm, limitation of motion, and, in advanced cases, muscle contractions and ankylosis

with permanent joint deformity. The most characteristic deformities in the hand are ulnar deviation of the fingers, the "boutonnière" deformity (flexion of the proximal interphalangeal joints and hyperextension of the distal interphalangeal joints resulting from volar slippage of the lateral band of superficial extensor tendons), and the "swan neck" deformity (hyperextension of the proximal interphalangeal joints and flexion of the distal interphalangeal joints resulting from contractures of intrinsic hand muscles).

3. Extra-articular manifestations–Twenty to 25% of patients (particularly those with severe disease) have subcutaneous or subperiosteal nodules, the so-called rheumatoid nodules. These are usually present over bony eminences, with the most common sites of nodule formation being the olecranon bursa and the extensor surface of the forearm. Nodules are firm, nontender, round or oval masses that can be movable or fixed. They may be found in the myocardium, pericardium, heart valves, pleura, lungs, sclera, dura mater, spleen, larynx, and synovial tissues.

Lung involvement includes pleurisy, interstitial lymphocytic pneumonitis or fibrosis, and Caplan's syndrome (development of large nodules in the lung parenchyma of patients with rheumatoid arthritis who also have pneumoconiosis). The manifestations of rheumatoid cardiac disease include pericarditis, myocarditis, valvular insufficiency, and conduction disturbances.

Several types of vasculitis occur in rheumatoid arthritis. The most common type is a small-vessel obliterative vasculitis that leads to peripheral neuropathy. Less common is a subacute vasculitis associated with ischemic ulceration of the skin. The rarest form of rheumatoid vasculitis is a necrotizing vasculitis of medium and large vessels indistinguishable from polyarteritis nodosa. The major neurologic abnormalities in rheumatoid arthritis involve peripheral nerves. In addition to the peripheral neuropathy associated with vasculitis, there are a number of entrapment syndromes due to impingement by periarticular inflammatory tissue or amyloid on nerves passing through tight fascial planes. The carpal tunnel syndrome is a well-known complication of wrist disease; however, entrapment can also occur at the elbow, knee, and ankle. Destruction of the transverse ligament of the odontoid results in atlantoaxial subluxation. This generally causes no symptoms but may be associated with cord or nerve root impingement.

Sjögren's syndrome (keratoconjunctivitis sicca and xerostomia) may occur in up to 30% of patients. Myositis with lymphocytic infiltration of involved muscle occurs rarely. Ocular involvement ranges from benign inflammation of the surface of the sclera (episcleritis) to severe inflammation of the sclera, with nodule formation. Scleronodular disease can lead to weakening and thinning of the sclera (scleromalacia). A catastrophic but rare complication of scleromalacia is perforation of the eye with extrusion of vitreous (scleromalacia perforans).

4. Felty's syndrome–Felty's syndrome is the association of rheumatoid arthritis, splenomegaly, and neutropenia. The syndrome almost always develops in patients with high rheumatoid factor titers and rheumatoid nodules, although the arthritis itself is frequently inactive. Other features of hypersplenism and lymphadenopathy may also be present. These patients are at increased risk of developing bacterial infections.

B. Laboratory Findings: A normochromic, normocytic anemia and thrombocytosis are common among patients with active disease. The sedimentation rate is elevated, and the degree of elevation correlates roughly with disease activity.

The synovial fluid is more inflammatory than that seen in degenerative osteoarthritis or SLE. The synovial fluid protein concentration ranges from 2.5 g/dL to over 3.5 g/dL. The white cell count is usually 5000–20,000/μL (rarely over 50,000/μL). Two-thirds of the cells are PMNs that discharge lysosomal enzymes into the synovial fluid, leading to depolymerization of synovial hyaluronate, decreased viscosity, and a poor mucin clot. The glucose level may be low or normal. Rheumatoid factor can be found in synovial fluid, and complement is often depressed.

The rheumatoid pleural effusion is an exudate containing less than 5000 mononuclear or polymorphonuclear leukocytes per microliter. Protein exceeds 3g/dL, and glucose is often reduced below 20 mg/dL. Rheumatoid factors can be detected, and complement levels are usually low.

Rheumatoid nodules consist of an irregularly shaped central zone of fibrinoid necrosis surrounded by a margin of large mononuclear cells with an outer zone of granulation tissue containing plasma cells and lymphocytes. They are thought to be a late stage in the evolution of a vasculitic process, probably induced by the deposition of circulating immune complexes.

C. X-Ray Findings: The first detectable x-ray abnormalities are soft tissue swelling and juxta-articular demineralization. The destruction of articular cartilage leads to joint space narrowing. Bony erosions develop at the junction of the synovial membrane and the "bare area" (unprotected cortical bone just adjacent to articular cartilage). Destruction of the cartilage and laxity of ligaments lead to maladjustment and subluxation of articular surfaces. Spondylitis is usually limited to the cervical spine, with osteoporosis, joint space narrowing, erosions, and finally subluxation of the involved articulations.

Immunologic Diagnosis

The most important serologic finding is the elevated rheumatoid factor titer, present in over 75% of patients. Rheumatoid factors are immunoglobulins with specificity for the Fc fragment of IgG. Most laboratory techniques detect 19S IgM rheumatoid factor, but rheumatoid factor properties are also seen in 7S IgM, IgG, and IgA immunoglobulins. 19S IgM rheumatoid factor may combine with IgG molecules to form a soluble circulating 22S immunoglobulin complex in the serum.

In rheumatoid arthritis, serum protein electrophoresis may show increased α_2-globulin, polyclonal hypergammaglobulinemia, and hypoalbuminemia. Cryoprecipitates composed of immunoglobulins are often seen in rheumatoid vasculitis. Serum complement levels are usually normal but may be low in the presence of active vasculitis. Twenty to 70% of patients have antinuclear antibodies.

Several tests are available in the laboratory to detect rheumatoid factor. The earliest test, now rarely used, was the streptococcal agglutination reaction. The latex fixation test is now the most commonly used method for detection of rheumatoid factor. Aggregated γ-globulin (Cohn fraction II) is adsorbed onto latex particles, which will then agglutinate in the presence of rheumatoid factor. The latex fixation test is not specific but is very sensitive, resulting in a high incidence of false-positive results. The sensitized sheep red cell test (Rose-Waaler test) depends on specific antibody binding and is the most specific test in common use. Sheep red blood cells are coated with rabbit antibody against sheep red blood cells. The sensitized sheep cells will then agglutinate in the presence of rheumatoid factor. More complicated tests include a radioimmunoassay for IgM rheumatoid factor and an immunodiffusion assay, which provide better quantification and more precise information on the immunoglobulin classes of rheumatoid factor.

It is important to emphasize that a negative rheumatoid factor by routine laboratory procedures does not exclude the diagnosis of rheumatoid arthritis. The so-called seronegative patient may have 7S IgG or IgM rheumatoid factor or circulating IgG-anti-IgG complexes. Conversely, rheumatoid factors are not unique to rheumatoid arthritis. Rheumatoid factor is also present in patients with SLE (30%), in a high percentage (90%) of patients with Sjögren's syndrome, and less often in patients with scleroderma or polymyositis. Positive agglutination reactions with the latex test also occur in patients with hypergammaglobulinemia associated with liver disease, kala-azar, sarcoidosis, and syphilis. The sensitized sheep red cell test is usually negative in these conditions. In some chronic infectious diseases such as leprosy and tuberculosis, both the latex and the sensitized sheep red cell tests may be positive. In subacute bacterial endocarditis, both tests may be positive during active disease and revert to negative as patients improve. The transient appearance of rheumatoid factor has been noted following vaccinations in military recruits. Epidemiologic studies have shown that a small number of normal people also have rheumatoid factors. A large proportion of the elderly have a positive latex test, though the sensitized sheep red cell test is generally negative.

Differential Diagnosis

In the patient with classic articular changes, bony erosions of the small joints of the hands and feet, and rheumatoid factor in serum or synovial fluid, the diagnosis of rheumatoid arthritis is not difficult. Early in the disease, or when extra-articular manifestations dominate the clinical picture, other rheumatic diseases (including SLE, Reiter's syndrome, gout, psoriatic arthritis, degenerative osteoarthritis, and the peripheral arthritis of chronic inflammatory bowel disease) or infectious processes may mimic rheumatoid arthritis. Patients with SLE can be distinguished by their characteristic skin lesions, renal disease, and diagnostic serologic abnormalities. Reiter's syndrome occurs predominantly in young men, generally affects joints of the lower extremity in an asymmetric fashion, and is often associated with urethritis and conjunctivitis. Gouty arthritis is usually an acute monoarthritis with negatively birefringent sodium urate crystals present within the white cells of inflammatory synovial fluid. Psoriatic arthritis often involves distal interphalangeal joints and produces nail changes. Degenerative arthritis is characterized by Heberden's nodes, lack of symmetric joint involvement, and involvement of the distal interphalangeal joints. The peripheral arthritis of bowel disease usually occurs in large weight-bearing joints and is often associated with bowel symptoms. The polyarthritis associated with rubella vaccination, HBsAg antigenemia, sarcoidosis, and infectious mononucleosis can mimic early rheumatoid arthritis.

Treatment

A. Physical Therapy: A rational program of physical therapy is vital in the management of patients with rheumatoid arthritis. Such a program should consist of an appropriate balance of rest and exercise and the judicious use of heat or cold therapy. The patient may require complete or intermittent bed rest on a regular basis to combat inflammation or fatigue. In addition, specific joints may have to be put at rest through the use of braces, splints, or crutches. An exercise program emphasizing active range-of-motion movements helps to maintain strength and mobility. Heat is valuable in alleviating muscle spasm, stiffness, and pain. Many patients need a hot shower or bath to loosen up in the morning, and others cannot perform their exercises adequately without prior heat treatment. Heating pads or paraffin baths are often used to apply heat to specific joints. Physical and occupational therapists provide valuable help in devising an appropriate physical therapy program.

B. Drug Treatment:

1. Salicylates—Salicylates are the mainstay of medical therapy of rheumatoid arthritis. Aspirin is an anti-inflammatory as well as an antipyretic and analgesic agent. Although its exact mechanism of action is uncertain, it may act in part by inhibiting the production of prostaglandins. The doses used to attain therapeutic levels (ie, 20–30 mg/dL) range from 3.6 to 6.5 g per day in divided doses. High-dosage aspirin therapy is associated with numerous side effects. Tinnitus—with or without hearing loss—is reversible with a decrease in dosage. Gastric distress is common but can be partly alleviated by liberally using antacids and by encouraging patients to take their aspirin with meals. Some patients can avoid gastric irritation by us-

ing enteric-coated aspirin. Microscopic blood loss from the gastrointestinal tract is common and is not an indication for stopping aspirin therapy. Since aspirin does decrease platelet adhesiveness, it should be used cautiously in patients with a bleeding diathesis or those receiving coumarin anticoagulants.

2. Other nonsteroidal agents—Several other nonsteroidal anti-inflammatory agents (fenoprofen, ibuprofen, naproxen, sulindac, tolmetin, mefenamic acid, and piroxicam) are useful in patients who cannot tolerate aspirin. Indomethacin may benefit some patients, but it has serious side effects (gastric intolerance, peptic ulceration, psychic disturbances, marrow depression). Combining 2 or more nonsteroidal anti-inflammatory agents provides little or no additional benefit over maximum doses of single agents.

3. Antimalarials—Many rheumatologists advocate the use of antimalarial drugs for prolonged periods in patients with severe disease. Their mechanism of action is unclear, but they appear to affect monocyte function. The antimalarials act slowly, often requiring 1–6 months of treatment for maximum therapeutic benefit. The preparations and dosages most often used are chloroquine, 250 mg orally daily, and hydroxychloroquine, 200–400 mg orally daily. The toxic side effects of these agents include skin rashes, nausea and vomiting, myopathy, and both corneal and retinal damage. The incidence of eye toxicity is rare at the low doses used in rheumatoid arthritis, but patients should have ophthalmologic examinations every 4–6 months while on antimalarial therapy.

4. Gold salt therapy—Although associated with a high incidence of toxic side effects, parenteral gold salt therapy is of significant benefit to many patients. It is one of the few therapeutic agents that is believed to alter the long-term course of the disease. Gold acts as a lysosomal membrane stabilizer and may chiefly affect macrophage function. It is administered intramuscularly, with an initial test dose of 10 mg of gold salt. If no toxic reactions occur after the test dose, the patient receives 50 mg of gold salt intramuscularly every week until clinical benefit ensues, at which time the interval between injections is gradually increased to every 3–4 weeks. If no benefit occurs after a total of 1500 mg has been administered, gold should be considered ineffective. Toxic side effects occur in 40% of patients and include dermatitis, photosensitivity, stomatitis, thrombocytopenia, agranulocytosis, hepatitis, aplastic anemia, peripheral neuropathy, nephritis with nephrotic syndrome, ulcerative enterocolitis, pneumonitis, and keratitis. Before each dose, the patient should be evaluated for possible toxic side effects. A urine protein measurement, hematocrit, white blood count, and platelet count should be obtained. Liver function tests should be performed periodically. Toxic side effects may necessitate temporary or permanent withdrawal of the drug. Corticosteroids and dimercaprol may be of benefit if life-threatening toxicity occurs.

An oral gold salt preparation, auranofin, is now available. Its main effect may be on T lymphocytes rather than on macrophages. Side effects, although similar to those of parenteral gold salt preparations, may occur less frequently. Diarrhea, however, is more common. The usual dose is 3 mg twice daily. As with parenteral gold preparations, it may take 3–6 months to achieve therapeutic benefit.

5. Penicillamine—Penicillamine is also useful in the treatment of rheumatoid arthritis. It may work through its inhibitory effect on helper T cell activity. Like gold, penicillamine is a slow-acting nonsteroidal anti-inflammatory agent, and it may take up to 6 months for a therapeutic response to become apparent. The incidence of drug toxicity is similar to that of parenteral gold, so during the initiation of therapy patients should be seen every other week for evaluation. Routine laboratory monitoring studies should include a complete blood count, platelet count, and urinalysis. The initial dose of 250 mg orally daily is increased by 125–250 mg every 4–12 weeks until improvement occurs or until the patient is receiving a maximum dose of 750 mg daily. Most patients require no more than 500 mg/d. Toxic side effects include rash, loss of sense of taste, nausea and vomiting, anorexia, proteinuria, agranulocytosis, aplastic anemia, and thrombocytopenia. Less commonly, myasthenia, myositis, Goodpasture's syndrome, pemphigus, bronchiolitis, and a lupuslike syndrome may be seen. Prior gold toxicity does not preclude the use of penicillamine.

6. Corticosteroids—Intermittent intra-articular injection of corticosteroids is useful for the patient with only a few symptomatic joints. Relief may last for months. However, multiple intra-articular corticosteroid injections in weight-bearing joints should be avoided, since they may lead to an increased incidence of degenerative arthritis. Systemic corticosteroids may induce a dramatic clinical response but should be used with extreme caution because of the many side effects associated with their long-term use. The usual dose is 5–10 mg of prednisone daily. Withdrawal from corticosteroids should be gradual, since clinical exacerbation of arthritis or steroid withdrawal syndrome may occur. Long-term systemic corticosteroid treatment results in hyperadrenocorticism and disruption of the pituitary-adrenal axis. Manifestations of corticosteroid toxicity include weight gain, moon facies, ecchymoses, hirsutism, diabetes mellitus, hypertension, osteoporosis, avascular necrosis of bone, cataracts, myopathy, mental disturbances, activation of tuberculosis, and infections.

7. Immunosuppressive agents—These have been known to induce dramatic improvement in patients with severe disease and, like gold, may alter the course of the disease. Alkylating agents (eg, chlorambucil, cyclophosphamide), purine analogs (eg, mercaptopurine, azathioprine), and antimetabolites (eg, methotrexate) have been used in the treatment of rheumatoid arthritis. However, these drugs are associated with major toxic side effects, may be teratogenic, and are associated with an increased incidence of neoplasm and infection. The routine use of these agents in

the treatment of rheumatoid arthritis is to be strongly discouraged.

C. Orthopedic Surgery: Surgery is often an essential part of the general management of the patient with rheumatoid arthritis. Surgical procedures can correct or compensate for joint damage. Arthroplasty is employed to maintain or improve joint motion. Arthrodesis can be used to correct deformity and alleviate pain, but it results in loss of motion. Early synovectomy might prevent joint damage or tendon rupture and will decrease pain and inflammation in a given joint, but the synovium often grows back and symptoms return.

Complications & Prognosis

Several clinical patterns of rheumatoid arthritis are apparent. Spontaneous remission may occur, usually within 2 years after the onset of the disease. Some patients have brief episodes of acute arthritis with longer periods of low-grade activity or remission. Rare patients will have sustained progression of active disease resulting in deformity and death. The development of classic disease within 1 year of the onset of symptoms, an age of less than 30 years at onset of disease, and the presence of rheumatoid nodules and high titers of rheumatoid factor are unfavorable prognostic factors.

Follow-up of patients after 10–15 years shows that 50% are stationary or improved, 70% are capable of full-time employment, and 10% are completely incapacitated. Death from vasculitis or atlantoaxial subluxation is rare. Fatalities are more often associated with sepsis or the complications of therapy.

JUVENILE ARTHRITIS

Juvenile arthritis is not a single disease but a group of disorders that cause arthritis in individuals under 16 years of age. It may present as a systemic illness (Still's disease) or as a seronegative polyarthritis. The prognosis in these instances is good. The incidence of the disease peaks in boys at age 2 and again at age 9, while in girls it peaks between 1 and 3 years of age. Less commonly, juvenile arthritis presents as a pauciarticular process involving 4 or fewer joints. The outlook for girls with pauciarticular disease is excellent, whereas boys with pauciarticular disease may eventually develop ankylosing spondylitis. In older children, juvenile arthritis occasionally presents as a seropositive polyarticular disease that follows a course identical to that of adult rheumatoid arthritis. Although upper respiratory infections and trauma have both been implicated as precipitating factors, the roles of infection, trauma, and heredity in the pathogenesis of the disease are unclear. Although the onset of the disease may be as early as 6 weeks of life, most children are between 2 and 5 or between 9 and 12 years of age at onset. Juvenile arthritis is a major cause of fever of undetermined origin in children.

Immunologic Pathogenesis

The basic immunopathogenic mechanisms in juvenile arthritis are unknown. However, both humoral and cellular defects occur in these patients. Diffuse hypergammaglobulinemia, involving IgG, IgA, and IgM, is present. Rheumatoid factors of all immunoglobulin classes have been detected. Approximately 10% of children with juvenile arthritis have a positive latex fixation test for 19S IgM rheumatoid factor. The sera from some patients with negative latex fixation tests may actually contain IgM rheumatoid factors. Two major theories have been offered in an attempt to explain the presence of these "hidden" rheumatoid factors in juvenile arthritis. First, IgM rheumatoid factor may bind avidly to native IgG in the patient's serum and therefore may not be able to bind IgG coating the latex particles. Second, an abnormal IgG may be present that preferentially binds IgM, thereby blocking latex fixation. Cold-reacting (4 °C) 19S IgM rheumatoid factors (cryoglobulins) are associated with severe disease.

Serum components of both the classic and alternative (properdin) complement systems are elevated, although this elevation is less in patients who have rheumatoid factors or severe disease. CH_{50}, C3, and C4 in the synovial fluid tend to be low, particularly in patients with a positive serum latex fixation or with IgG rheumatoid factor in the synovial fluid. Elevation of serum complement may reflect a secondary overcompensation in response to increased consumption, or possibly a general increase in protein synthesis. Studies of the metabolism of complement actually demonstrate hypercatabolism. The depression of complement in synovial fluid is probably secondary to complement activation by immune complexes, similar to that seen in rheumatoid arthritis.

Preliminary studies suggest that patients with juvenile arthritis possess certain HLA tissue types with greater than expected frequencies. Thus, patients with early-onset pauciarticular disease tend to be HLA-DR5- or HLA-DR8-positive, while those with late-onset pauciarticular disease tend to be HLA-B27-positive. Patients with rheumatoid factor-positive polyarticular disease tend to be HLA-D4-positive, and those with systemic disease tend to be HLA-DR5-positive.

Clinical Features

A. Symptoms and Signs:

1. Onset–

a. Twenty percent of children, usually under age 4, present with high, spiking fever, an evanescent rash, polyserositis, hepatosplenomegaly, and lymphadenopathy (Still's disease).

b. Forty percent of patients present with polyarthritis (more than 4 joints involved), sometimes accompanied by low-grade fever and malaise. In 25% of this group, the onset is in late childhood and is associated with rheumatoid factor.

c. Forty percent of patients present with few systemic manifestations and asymmetric involvement of only one or 2 joints. Slightly more than 50% of these

patients are young girls particularly likely to develop iridocyclitis.

2. Joint manifestations–Even in the presence of severe arthritis, young children may not complain of pain but may instead limit the use of an extremity. The knees, wrists, ankles, and neck are common sites of initial involvement. Early involvement of the hip is extremely rare in young children with pauciarticular disease. Older children occasionally develop symmetric involvement in the small joints of the hands (metacarpophalangeal, proximal interphalangeal, and distal interphalangeal) similar to that seen in adults. In seronegative patients, the metacarpophalangeal joints may be spared. With severe hand involvement, children are more likely to develop radial rather than ulnar deviation. Involvement of the feet may lead to hallux valgus or hammer toe deformity. Achillobursitis and achillotendinitis may cause tender, swollen heels. Older boys with pauciarticular disease commonly develop ankylosing spondylitis.

3. Systemic manifestations–Fever, often with a high evening spike, is characteristic of Still's disease. Anorexia, weight loss, and malaise are common. Most children with Still's disease develop an evanescent, salmon-colored maculopapular rash that coincides with periods of high fever. Occasional patients manifest cardiac involvement. Pericarditis is the most common cardiac manifestation but rarely leads to dysfunction or constriction. Myocarditis is an unusual manifestation of the cardiac disease, but heart failure occurs occasionally.

Cases of acute pneumonitis or pleuritis have been described. However, chronic rheumatoid lung disease is rarely seen in juvenile arthritis.

Iridocyclitis occurs most commonly in young girls with pauciarticular disease. This manifestation rarely precedes articular involvement, but it often persists even when joints become quiescent. The iridocyclitis often runs an insidious course and is best monitored by frequent slit lamp examinations, at least through puberty.

Lymphadenopathy and hepatosplenomegaly are associated with severe systemic disease and are uncommon in patients with chiefly articular manifestations.

Subcutaneous nodules occur in children with polyarticular disease, usually in association with a positive test for rheumatoid factor.

Rarely, Still's disease occurs in adults. Characteristic manifestations include high spiking fevers, evanescent rash, arthritis, and elevated white blood cell count and hepatic enzyme levels.

4. Complications–The major complication of juvenile arthritis is impairment of growth and development secondary to early epiphyseal closure. This is particularly common in the mandible, causing micrognathia, and in the metacarpals and metatarsals, leading to abnormally small fingers and toes. The extent of growth impairment usually correlates positively with the severity and duration of disease but may also reflect the growth-inhibiting effects of steroids. Vasculitis and encephalitis are occasionally observed in patients with juvenile arthritis. Secondary amyloidosis occurs rarely.

B. Laboratory Findings: Mild leukocytosis (15,000–20,000/μL) is the rule, but some patients develop leukopenia. A normochromic microcytic anemia, an elevated erythrocyte sedimentation rate, and an abnormal C-reactive protein occur commonly. Because an elevated ASO titer is so frequently encountered, this test cannot be used to differentiate juvenile arthritis from rheumatic fever. Positive tests for rheumatoid factor occur in older children with polyarticular disease, while antinuclear antibodies are found both in patients with polyarticular disease and in young patients with pauciarticular disease. Antinuclear antibodies almost never occur in Still's disease. Serum protein electrophoresis shows an increase in acute phase reactants (alpha globulins) and a polyclonal increase of γ-globulin. The synovial fluid in active juvenile rheumatoid arthritis is exudative, with a white count of 5000–20,000/μL (mostly neutrophils), a poor mucin clot, and decreased glucose compared to serum glucose.

C. X-Ray Findings: Radiographic changes early in the disease include periosteal bone accretion, premature closure of the epiphyses, cervical zygapophyseal fusion (particularly at C2–3), and osseous overgrowth of the interphalangeal joints, resembling Heberden's or Bouchard's nodes. Late findings include juxta-articular demineralization and erosion and narrowing of the joint space. Carpal arthritis with ankylosis is seen as a late manifestation of Still's disease.

Immunologic Diagnosis

Currently, the diagnosis of juvenile arthritis is based on clinical criteria. Although certain abnormalities of immunoglobulins, complement, and cellular immunity are compatible with the diagnosis of juvenile arthritis, no specific immunologic test is available.

Differential Diagnosis

The diagnosis of juvenile arthritis is extremely difficult, since the disease can present with nonspecific constitutional signs and symptoms in the absence of arthritis. Other causes of fever, particularly infections and cancer, must be considered. Leukemia can present in childhood with fever, lymphadenopathy, and joint pains. Rheumatic fever closely resembles juvenile arthritis, particularly early in the disease. However, the patient with juvenile arthritis tends to have higher spiking fevers, lymphadenopathy and hepatosplenomegaly in the absence of carditis, and a more refractory, long-lasting arthritis. Rheumatic fever patients are more likely to have evidence of recent streptococcal infection, including elevated titers of antihyaluronidase, antistreptokinase, and antistreptodornase. In addition, patients with rheumatic fever tend to have a less intense leukocytosis and respond more dramatically to low doses of salicylates. An expanding skin lesion followed in weeks or months

by arthritis suggests the diagnosis of Lyme disease, an inflammatory arthropathy caused by the spirochete *Borrelia burgdorferi*. Rheumatic diseases that may begin in childhood, such as SLE or dermatomyositis, can be differentiated by their different clinical course, different organ system involvement, and characteristic serologic abnormalities.

When juvenile arthritis presents primarily with arthritis, examination of synovial fluid is of paramount importance in excluding infection.

Treatment

The major goals of therapy are to relieve pain, prevent contractures and deformities, and promote normal emotional and physical development. These goals are best achieved by a comprehensive program of physical, medical, and, when necessary, surgical therapy.

A. Physical Therapy: As in the treatment of adult rheumatoid arthritis, rest is an important part of physical therapy. Complete rest is indicated during exacerbations and may be necessary for short afternoon periods on a routine basis. Specific joints can be put at rest by the use of splints, collars, and braces that support the joint and help prevent deformity. Judiciously used heat will decrease pain and muscle spasm and is particularly useful before exercising. Exercise promotes muscle strength, encourages growth, and prevents deformity.

B. Medical Therapy:

1. Aspirin—The disease responds to aspirin at a dosage level of 90–130 mg/kg/d given in 4–6 divided doses. Tinnitus and decreased hearing are poor indicators of aspirin toxicity in children. Irritability, drowsiness, or intermittent periods of hyperpnea are early signs of salicylate intoxication. Therefore, it is essential to monitor blood salicylate levels during aspirin therapy. Acidosis and ketosis may develop in infants. Respiratory alkalosis, due to primary stimulation of the respiratory center, occurs in older children.

2. Gold salts—Gold salts should be considered in children with polyarticular disease who are unresponsive to salicylates. If a test dose of 5 mg intramuscularly does not result in toxic signs, 1 mg/kg/wk intramuscularly should be given. Best results are obtained when the drug is used early in the course of the disease.

3. Corticosteroids—Intra-articular corticosteroid injections are useful in pauciarticular disease. Systemic corticosteroids are reserved for patients with myocarditis, vasculitis, refractory iridocyclitis, or Still's disease that is unresponsive to aspirin therapy. Patients with iridocyclitis may require prolonged corticosteroid therapy. In children, the major toxic effects of corticosteroid therapy include subcapsular cataract formation, vertebral osteoporosis and collapse, infection, premature skeletal maturation with diminished growth, and pseudotumor cerebri with intracranial hypertension.

C. Surgical Treatment: The aims of surgery in juvenile arthritis are to relieve pain and maintain or improve joint function. Synovectomy may diminish pain due to chronic synovitis, but long-term effectiveness is questionable. Synovectomy for severe extensor tenosynovitis of the hand may prevent tendon rupture. Tendon release procedures help relieve joint contractures. Hip replacement is of benefit in selected cases but should be delayed as long as possible, since in some children hip cartilage may regenerate with continued weight bearing.

Complications & Prognosis

Seventy percent of patients experience a spontaneous and permanent remission by adulthood. Patients with Still's disease tend to have 1–10 recurrences per year. Patients presenting with oligoarthritic disease, particularly if they are female, tend to remain oligoarthritic, while those presenting with polyarthritis remain polyarthritic. Rarely, the disease persists into adulthood. This usually occurs in children with symmetric polyarthritis similar to that seen in adults. Sometimes a patient with juvenile arthritis in apparent remission develops rheumatoid arthritis as an adult. In an occasional unfortunate case, the disease is relentless and crippling. Small joint involvement, positive serum rheumatoid factor, and onset in later childhood all portend a poor prognosis.

SJÖGREN'S SYNDROME

Major Immunologic Features

- Lymphocyte and plasma cell infiltration of involved tissues.
- Hypergammaglobulinemia, rheumatoid factor, and antinuclear antibodies, including specific acid-extractable nuclear antigens.
- Autoantibodies against salivary duct antigens.

General Considerations

Sjögren's syndrome is a chronic inflammatory disease of unknown cause characterized by diminished lacrimal and salivary gland secretion resulting in keratoconjunctivitis sicca and xerostomia. There is dryness of the eyes, mouth, nose, trachea, bronchi, vagina, and skin. In half of patients, the disease occurs as a primary pathologic entity (primary Sjögren's syndrome). In the other half, it occurs in association with rheumatoid arthritis or other connective tissue disorders. Ninety percent of patients with Sjögren's syndrome are female. Although the mean age at onset is 50 years, the disease has been detected in children.

Immunologic Pathogenesis

The strong association of Sjögren's syndrome with rheumatoid arthritis and SLE suggests that immunologic processes play a role in the pathogenesis of this disease. It has been hypothesized that patients with Sjögren's syndrome respond abnormally to one or more unidentified antigens, perhaps viral antigens or virus-altered autoantigens. This abnormal response is characterized by excessive B cell and plasma cell ac-

tivity, manifested by polyclonal hypergammaglobulinemia and the production of rheumatoid factor, antinuclear factors, cryoglobulins, and anti-salivary duct antibodies. Immunofluorescence studies have shown both B and T lymphocytes and plasma cells infiltrating involved tissues. Large quantities of IgM and IgG are synthesized by these infiltrating lymphocytes. In patients with coexisting macroglobulinemia, monoclonal IgM may be synthesized in the salivary glands. Excessive B cell activity could be due either to a primary B cell defect or to defective T lymphocyte regulation, since there is evidence of decreased suppressor T cell function in patients with Sjögren's syndrome.

Clinical Features

A. Symptoms and Signs:

1. Oral–Dryness of the mouth is usually the most distressing symptom and is often associated with burning discomfort and difficulty in chewing and swallowing dry foods. Polyuria and nocturia develop as the patient drinks increasing amounts of water in an effort to relieve these symptoms. The oral mucous membranes are dry and erythematous, and the tongue becomes fissured and ulcerated. Severe dental caries is often present. Half of patients have intermittent parotid gland enlargement with rapid fluctuations in the size of the gland. The parotid gland in Sjögren's syndrome is firm in contrast to the soft parotid enlargement characteristic of diabetes mellitus or alcohol abuse. Oral candidiasis can be a complication of Sjögren's syndrome.

2. Ocular–The major ocular finding is keratoconjunctivitis sicca. Symptoms include burning, itching, decreased tearing, ocular accumulation of thick mucoid material during the night, photophobia, pain, and a "gritty" or "sandy" sensation in the eyes. Decreased tearing is demonstrated by an abnormal Schirmer test. Slit lamp examination reveals punctate rose bengal or fluorescein staining of the conjunctiva and cornea, strands of corneal debris, and a shortened tear film break-up time. Severe ocular involvement may lead to corneal ulceration, vascularization with opacification, or perforation.

3. Miscellaneous–Dryness of the nose, posterior oropharynx, larynx, and respiratory tract may lead to epistaxis, dysphonia, recurrent otitis media, tracheobronchitis, or pneumonia. The vaginal mucosa is also dry, and women commonly complain of dyspareunia. Active synovitis is a common finding, particularly in patients who also have rheumatoid arthritis. Twenty percent of patients with primary Sjögren's syndrome complain of Raynaud's phenomenon. Ten percent of patients have extraglandular lymphocytic infiltrates, particularly in the kidneys, lungs, lymph nodes, and muscles. A few such patients may develop lymphoma.

B. Laboratory Findings: Anemia, leukopenia, and an elevated erythrocyte sedimentation rate are common features. Parotid salivary flow is less than the normal 5 mL/10 min/gland. Secretory sialography with radiopaque dye demonstrates many findings of glandular disorganization. Salivary scintigraphy with technetium Tc 99m pertechnetate reveals decreased parotid secretory function. Histologically, a lymphocytic infiltrate involves exocrine glands of the respiratory, gastrointestinal, and vaginal tracts as well as glands of the ocular and oral mucosa. Histologic demonstration of lymphocytic infiltration in a biopsy specimen taken from the minor labial salivary glands is the most specific and sensitive single diagnostic test for Sjögren's syndrome.

Immunologic Diagnosis

No immunologic test is diagnostic for Sjögren's syndrome. However, a myriad of nonspecific immunologic abnormalities occur in these patients.

A. Humoral Abnormalities: Hypergammaglobulinemia is seen in half of patients. Although serum protein electrophoresis usually shows a polyclonal hypergammaglobulinemia, occasional patients develop a monoclonal IgM paraproteinemia, usually of the kappa type. Patients who develop lymphoma sometimes become severely hypogammaglobulinemic and show disappearance of autoantibodies. Rheumatoid factors can be detected by latex fixation in 90% of patients with Sjögren's syndrome. ANA in a speckled or homogeneous pattern is present in 70% of patients. Many of these antinuclear antibodies are directed against acid-extractable nuclear antigens. Antibodies against one such antigen, termed SS-B, are relatively specific for patients with primary Sjögren's syndrome. Antibodies against a second acid-extractable nuclear antigen, SS-A, may be found in Sjögren's syndrome alone or in Sjögren's syndrome associated with systemic lupus erythematosus. Patients with Sjögren's syndrome and rheumatoid arthritis have neither anti-SS-A nor anti-SS-B antibodies. They tend, instead, to develop antibodies against the Epstein-Barr virus-associated nuclear antigen called rheumatoid arthritis nuclear antigen (RANA). Autoantibodies against salivary duct antigens have been detected in 50% of patients with Sjögren's syndrome associated with rheumatoid arthritis.

B. Cellular Abnormalities: Thirty percent of patients with Sjögren's syndrome have decreased lymphocyte responses to mitogenic stimulation. A few patients also have decreased numbers of circulating T lymphocytes in the peripheral blood (see Immunologic Pathogenesis, above).

C. HLA Associations: HLA typing studies suggest a genetic predisposition to the development of Sjögren's syndrome. The prevalence of both HLA-DR3 and HLA-B8 is increased in patients with primary Sjögren's syndrome and Sjögren's syndrome with SLE.

Differential Diagnosis

The diagnosis of Sjögren's syndrome can be made on the basis of 2 of the 3 classic manifestations (xerostomia, keratoconjunctivitis sicca, and rheumatoid arthritis). However, the varied and multisystemic nature of the disease may obscure the diagnosis. Certainly, any patient with a rheumatic disease—eg,

SLE, rheumatoid arthritis, or scleroderma—should be observed for Sjögren's syndrome; likewise, any patient with Sjögren's syndrome should be examined for the purpose of ruling out other rheumatic diseases. Other causes of parotid swelling include nutritional deficiencies, endocrine disorders, sarcoidosis, drug reactions, infections, and obesity. Parotid gland cancer must always be considered in a patient with unilateral parotid swelling.

Treatment

A. Symptomatic Measures:

1. Oral–Patients must be urged to maintain fastidious oral hygiene, with regular use of fluoride toothpaste, mouthwashes, and regular dental examinations. Frequent sips of water and the use of sugarless gum or candy to stimulate salivary secretion are sometimes helpful in relieving xerostomia. Many patients find aerosolized preparations of artificial saliva helpful. A bedroom humidifier will help decrease nocturnal xerostomia and nasal dryness.

2. Ocular–Methylcellulose artificial tears alleviate ocular symptoms and protect against ocular complications. Shielded glasses offer protection against the drying effects of wind. Therapy for refractory ocular complications includes mucolytic agents, punctal occlusion, soft contact lenses, and partial tarsorrhaphy.

B. Systemic Measures: Sjögren's syndrome can usually be controlled with symptomatic therapy. Fast-acting nonsteroidal anti-inflammatory agents are useful in the treatment of the nonerosive arthritis of Sjögren's syndrome. In severe or life-threatening disease, corticosteroids or immunosuppressive agents have been used. These drugs are indicated primarily in patients with lymphoma, Waldenström's macroglobulinemia, or massive lymphocytic infiltration of vital organs (such as the lung).

Complications & Prognosis

In the vast majority of patients, significant lymphoproliferation is confined to salivary, lacrimal, and other mucosal glandular tissue, resulting in a benign chronic course of xerostomia and xerophthalmia. Rarely, patients develop significant extraglandular lymphoid infiltration or neoplasia.

Splenomegaly, leukopenia, and vasculitis with leg ulcers may occur. Hypergammaglobulinemic purpura, often associated with renal tubular acidosis, has been described and may be a presenting complaint. Five percent of patients with Sjögren's syndrome develop chronic autoimmune thyroiditis. Other associations include primary biliary cirrhosis, chronic active hepatitis, gastric achlorhydria, pancreatitis, renal and pulmonary lymphocytic infiltration, cryoglobulinemia with glomerulonephritis, hyperviscosity syndrome, and adult celiac disease. Neuromuscular complications include polymyositis, peripheral or cranial (particularly trigeminal) neuropathy, and cerebral vasculitis. Rarely, patients with Sjögren's syndrome develop lymphoid cancer, immunoblastic sarcoma, or Waldenström's macroglobulinemia. The lymphoma is often a monoclonal B cell neoplasm containing intracellular IgM-κ immunoglobulin.

PROGRESSIVE SYSTEMIC SCLEROSIS

Major Immunologic Features

- Antinuclear antibodies with a speckled or nucleolar pattern.
- Anticentromere antibodies in patients with CREST syndrome.
- Antibodies against an acid-extractable nuclear antigen in progressive systemic sclerosis.

General Considerations

Progressive systemic sclerosis is a disease of unknown cause characterized by abnormally increased collagen deposition in the skin. The course is usually slowly progressive and chronically disabling, but it can be rapidly progressive and fatal because of involvement of internal organs. It commonly begins in the third or fourth decade of life. Children are occasionally affected. The incidence of the disease is 4–12.5 cases per million population. Women are affected twice as often as men, and there is no racial predisposition.

Immunologic Pathogenesis

The association of progressive systemic sclerosis with Sjögren's syndrome and, less often, with thyroiditis—and the serologic abnormalities seen in the majority of cases (presence of ANA, rheumatoid factor, polyclonal hypergammaglobulinemia)—are suggestive of an immunologic aberration in these patients. At present, there is scanty evidence for a humoral mechanism in the pathogenesis of the disease, although a serum factor toxic to vascular endothelium has been identified. Immunoglobulins have not been found at the dermal-epidermal junction in scleroderma, although examination of the fibrinoid lesions seen in the walls of renal arterioles has revealed the presence of γ-globulin and complement. The ability of lymphocytes to destroy embryonic fibroblasts in tissue cultures may indicate an alteration in cellular immunity in these patients. However, in contrast to other autoimmune diseases, cellular infiltration in scleroderma is minimal or absent in all organs except the synovium, where impressive collections of lymphocytes and plasma cells can be seen. Unfortunately, research on the pathogenesis of progressive systemic sclerosis is severely hampered by the absence of an animal model.

Clinical Features

A. Symptoms and Signs:

1. Onset–In more than half of cases, Raynaud's phenomenon heralds the onset of the disease. Progressive systemic sclerosis frequently begins with skin changes, but in one-third of patients polyarthralgias

and polyarthritis are the first manifestations. Initial visceral involvement without skin manifestations is very unusual.

2. Skin abnormalities–There are 3 stages in the clinical evolution of scleroderma. In the edematous phase, symmetric nonpitting edema is present in the hands and, rarely, in the feet. The edema can progress to the forearms, arms, upper anterior chest, abdomen, back, and face. In the sclerotic phase, the skin is tight, smooth, and waxy and seems bound down to underlying structures. Skin folds and wrinkles disappear. The hands are involved in most patients, with painful, slowly healing ulcerations of the fingertips in half of those cases. The face appears stretched and masklike, with thin lips and a "pinched" nose. Pigmentary changes and telangiectases are frequent findings at this stage. The skin changes may stabilize for prolonged periods and then either progress to the third (atrophic) stage or soften and return to normal. It should be emphasized that not all patients pass through all the stages. Subcutaneous calcifications, usually in the fingertips (calcinosis circumscripta), occur more often in women than in men. The calcifications vary in size from tiny deposits to large masses and may develop over bony prominences throughout the body.

3. Raynaud's phenomenon–Raynaud's phenomenon occurs in over 90% of patients. In the so-called CREST syndrome—*c*alcinosis, *R*aynaud's phenomenon, *e*sophageal dysmotility, *s*clerodactyly, and *t*elangiectases—the disease may remain confined to the fingers for prolonged periods.

4. Joints and muscles–Articular complaints are very common in progressive systemic sclerosis and may begin at any time during the course of the disease. The arthralgias, stiffness, and frank arthritis seen in progressive systemic sclerosis may be difficult to distinguish from those of rheumatoid arthritis, particularly in the early stages of the disease. Involved joints include the metacarpophalangeals, proximal interphalangeals, wrists, elbows, knees, ankles, and small joints of the feet. Flexion contractures, due to changes in the skin or joints, are common. Muscle involvement is usually mild but may be clinically indistinguishable from that of polymyositis, with muscle weakness, tenderness, and pain of proximal muscles of the upper and lower extremities.

5. Lungs–The lungs are frequently involved in progressive systemic sclerosis, either clinically or at autopsy. A low diffusion capacity is the earliest detectable abnormality, preceding alterations in ventilation or clinical and radiologic evidence of disease. Dyspnea on exertion is the most frequently reported symptom. Orthopnea, paroxysmal nocturnal dyspnea, chronic cough, hemoptysis, chest pain, and hoarseness are also manifestations of pulmonary involvement. Pleurisy (with associated pleural friction rub) can also occur. Pulmonary fibrosis, often associated with pulmonary hypertension, is a recognized complication of CREST syndrome. Patients with diffuse pulmonary involvement have intimal proliferation of small- and medium-sized pulmonary arteries and arterioles and may have an intense bronchiolar epithelial proliferation.

6. Heart–Because of the frequency of pulmonary fibrosis, cor pulmonale is the commonest cardiac finding in progressive systemic sclerosis. Myocardial fibrosis, leading to digitalis-resistant left-sided heart failure, carries a poor prognosis. Cardiac arrhythmias and conduction disturbances are common manifestations of myocardial fibrosis. The pericarditis of progressive systemic sclerosis is usually asymptomatic and is found incidentally at autopsy. Although 40% of patients have pericardial effusion by electrocardiography, tamponade is extremely rare.

7. Kidneys–Renal involvement is an uncommon but life-threatening development in progressive systemic sclerosis. Although renal insufficiency may follow an indolent course, it frequently presents as rapidly progressive oliguric renal failure with or without malignant hypertension.

8. Gastrointestinal tract–The gastrointestinal tract is commonly affected in progressive systemic sclerosis. The esophagus is the most frequent site of involvement, with dysphagia or symptoms of reflux esophagitis occurring in 80% of patients. Gastric and small bowel involvement presents with cramping, bloating, and diarrhea alternating with constipation. Hypomotility of the gastrointestinal tract with bacterial overgrowth may result in malabsorption. Colonic scleroderma is associated with chronic constipation.

9. Sjögren's syndrome–Sicca syndrome is seen in 5–7% of patients.

10. Uncommon clinical manifestations–Biliary cirrhosis or mononeuropathy, either cranial or peripheral, may rarely be associated with progressive systemic sclerosis.

11. Mixed connective tissue disease–Mixed connective tissue disease is a syndrome with features of scleroderma, rheumatoid arthritis, SLE, and polymyositis-dermatomyositis. The manifestations of the disease include arthritis, Raynaud's phenomenon, scleroderma of the fingers, muscle weakness and tenderness, interstitial lung disease, and a skin rash resembling either dermatomyositis or SLE. These patients have a high-titer speckled pattern of ANA and antibody to the ribonuclease-sensitive component of extractable nuclear antigen. Renal disease is unusual in these patients. The disease appears to respond to moderate doses of corticosteroids.

B. Laboratory Findings: The normochromic normocytic anemia of chronic inflammatory disease is occasionally seen in progressive systemic sclerosis. Microangiopathic anemia can also occur. An elevated erythrocyte sedimentation rate and polyclonal hypergammaglobulinemia are common. A positive speckled or nucleolar pattern ANA is frequently encountered.

Biopsy of clinically involved skin reveals thinning of the epidermis with loss of the rete pegs, atrophy of the dermal appendages, hyalinization and fibrosis of arterioles, and a striking increase of compact collagen fibers in the reticular dermis.

Synovial tissue findings range from an acute inflammatory lymphocytic infiltration to diffuse fibrosis with relatively little inflammation.

The histologic changes seen in muscle tissue include interstitial and perivascular inflammatory infiltration followed by fibrosis and myofibrillar necrosis, atrophy, and degeneration.

In patients with renal involvement, the histologic appearance of the kidney is similar to that of malignant hypertensive nephropathy, with intimal proliferation of the interlobular arteries and fibrinoid changes in the intima and media of more distal interlobular arteries and of afferent arterioles.

There is increased collagen deposition in the lamina propria, submucosa, and muscularis of the gastrointestinal tract. Small-vessel changes similar to those that occur in the skin may also result. With loss of normal smooth muscle, the large bowel is subject to development of the characteristic wide-mouthed diverticula and to infiltration of air into the wall of the intestine (pneumatosis cystoides intestinalis).

C. X-Ray Findings:

1. Bone x-rays—Thickening of the periarticular soft tissues and juxta-articular osteoporosis are seen in involved joints. Absorption of the terminal phalanges is often associated with soft tissue atrophy and subcutaneous calcinosis.

2. Chest x-rays—Characteristically, a diffuse increase in interstitial markings is seen in the lower lung fields of patients with moderate to severe pulmonary involvement. "Honeycombing," nodular densities, and disseminated pulmonary calcifications may also be seen.

3. Gastrointestinal x-rays—Upper gastrointestinal series will often reveal decreased or absent esophageal peristaltic activity, even in patients without symptoms of dysphagia. Long-standing disease leads to marked dilation of the lower two-thirds of the esophagus. Gastrointestinal reflux is present in the majority of cases, and ulcers or strictures of the lower esophagus due to peptic esophagitis are commonplace. With gastrointestinal involvement, barium is often retained in the second and third portions of the duodenum. Intestinal loops become dilated and atonic, with irregular flocculation and hypersegmentation.

The barium enema may reveal large, wide-mouthed diverticula along the antimesenteric border of the colon.

4. Renal arteriography—Marked changes are seen on renal arteriography in patients with scleroderma kidney. Irregular arterial narrowing, tortuosity of the interlobular arterioles, persistence of the arterial phase, and absence of a nephrogram phase are typical findings.

Immunologic Diagnosis

Polyclonal hypergammaglobulinemia is a frequent serologic abnormality in progressive systemic sclerosis. The fluorescent antinuclear antibody test shows a speckled or nucleolar pattern in 70% of cases. A specific antinuclear antibody found only in patients with progressive systemic sclerosis has been identified. Many patients with CREST syndrome have a positive ANA owing to the presence of anticentromere antibodies.

Differential Diagnosis

When classic skin changes and Raynaud's phenomenon are associated with characteristic visceral complaints, the diagnosis is obvious. In patients presenting with visceral or arthritic complaints and no skin changes, the diagnosis is difficult. In many cases, only the presence or absence of antibodies to ribonuclease-sensitive extractable nuclear antigen makes it possible to differentiate scleroderma from mixed connective tissue disease. Patients with eosinophilic fasciitis present with marked thickening of the skin similar to that seen in the edematous phase of scleroderma. However, Raynaud's phenomenon and visceral involvement are rare in eosinophilic fasciitis, and fibrosis and inflammatory cell infiltration are seen in the deep fascial layers, whereas in scleroderma the fibrosis occurs predominantly in the dermis. The differential diagnosis also includes scleromyxedema, polyvinyl chloride exposure, carcinoid syndrome, phenylketonuria, porphyria cutanea tarda, amyloidosis, Werner's syndrome, and progeria.

Treatment

There is at present no cure for progressive systemic sclerosis. Sympathectomy has resulted in only transient relief of vascular symptoms, but vasodilating agents, particularly calcium channel-blockers, have provided relief for patients with severe Raynaud's phenomenon. Corticosteroids have no effect on the visceral progression of the disease, though they are beneficial in scleroderma with myositis and in mixed connective tissue disease. Colchicine has limited efficacy in treatment of the cutaneous manifestations of the disease. Penicillamine is often effective in the treatment of cutaneous scleroderma, and evidence suggests that it may be of benefit in slowing the progression of visceral disease.

Patients should avoid exposure to cold and should wear gloves to protect their hands. Tobacco should be avoided. Skin ulcers require careful antiseptic care. Cor pulmonale and left-sided heart failure may be treated with diuretics and digitalization, although the response is often poor. Antibiotics may be beneficial in decreasing intestinal bacterial overgrowth that leads to malabsorption.

Hypertensive crisis in renal disease associated with progressive systemic sclerosis is very difficult to control even with potent hypotensive agents. Captopril, an inhibitor of angiotensin-converting enzyme, may be of benefit in treating the renal disease associated with scleroderma. The arthritis can usually be controlled with aspirin and other fast-acting nonsteroidal anti-inflammatory drugs. Skin lubricants can alleviate dryness and cracking.

Complications & Prognosis

Spontaneous remissions occur in progressive systemic sclerosis, but the usual course of the disease is one of relentless progression from dermal to visceral involvement. Involvement of the heart, lung, or kidney is associated with a high mortality rate. Aspiration pneumonia resulting from esophageal dysfunction is a complication in advanced disease.

Although the prognosis for any given patient is extremely variable, the overall 5-year survival rate for progressive systemic sclerosis is approximately 40%.

POLYMYOSITIS-DERMATOMYOSITIS

Major Immunologic Features

- Production of cytotoxin by lymphocytes incubated with autologous muscle.
- Lymphocytic and plasma cell infiltration of involved muscle.
- Antibodies to the nuclear antigens Jo-1, PM-Scl, and RNP.

General Considerations

Polymyositis-dermatomyositis is an acute or chronic inflammatory disease of muscle and skin that may occur at any age. Women are affected twice as commonly as men. There is no racial preponderance. The incidence of the disease is one per 200,000 population.

Polymyositis-dermatomyositis can be subclassified into 5 categories: (1) idiopathic polymyositis, (2) idiopathic dermatomyositis, (3) polymyositis-dermatomyositis associated with cancer, (4) childhood polymyositis-dermatomyositis, and (5) polymyositis-dermatomyositis associated with other rheumatic diseases (Sjögren's syndrome, SLE, progressive systemic sclerosis, mixed connective tissue disease).

Immunologic Pathogenesis

Although the precise pathogenetic mechanisms are unknown, there is a great deal of evidence that autoimmunity may play a role in disease causation. Experimental polymyositis has been induced in rats and guinea pigs by the injection of allogeneic muscle tissue in Freund's complete adjuvant. Polymyositis-dermatomyositis may coexist with other autoimmune diseases. An infectious cause has been suggested, but no microorganism has ever been conclusively implicated.

A. Humoral Factors: Polyclonal hypergammaglobulinemia is common in patients with polymyositis-dermatomyositis, and rheumatoid factors and antinuclear antibodies occur in 20% of cases. In children, focal deposits of complement, IgG, and IgM have been seen in vessel walls of involved skin and muscle. Some patients with polymyositis-dermatomyositis have been shown to produce antibodies both against a component of the extractable nuclear antigen and against purified human skeletal muscle myoglobin.

B. Cellular Factors: There is evidence that cellular immunity plays a role in the pathogenesis of polymyositis-dermatomyositis. Polymyositis has been induced in rats and guinea pigs by the transfer of sensitized lymphoid cells. Lymphocytes from patients with polymyositis-dermatomyositis, after incubation with normal autologous muscle, produce a lymphokine that is toxic to monolayers of human fetal muscle cells. The lymphocytes in the muscle infiltrate of patients with polymyositis-dermatomyositis produce this lymphotoxin upon simple incubation of involved muscle. Thus, the lymphocytes of patients with polymyositis-dermatomyositis may respond to their own muscle antigens as if they were foreign (Fig 21–3). It is not known whether this is a primary defect in antigen recognition by the lymphocytes or whether these muscle antigens are cross-reactive with an unidentified foreign antigen.

Clinical Features

A. Symptoms and Signs:

1. Onset–Although the symptoms may begin abruptly, the onset of the disease is usually insidious.

2. Muscle involvement–The commonest manifestation is weakness of involved striated muscle. The proximal muscles of the extremities are most often affected, usually progressing from the lower to the upper limbs. The distal musculature is involved in only 25% of patients. Weakness of the cervical muscles with inability to raise the head and weakness of the posterior pharyngeal muscles with dysphagia and dysphonia are also seen. Facial and extraocular muscle involvement is unusual. Muscle pain, tenderness, and edema may be seen.

3. Skin involvement–The characteristic rash of dermatomyositis, present in approximately 40% of pa-

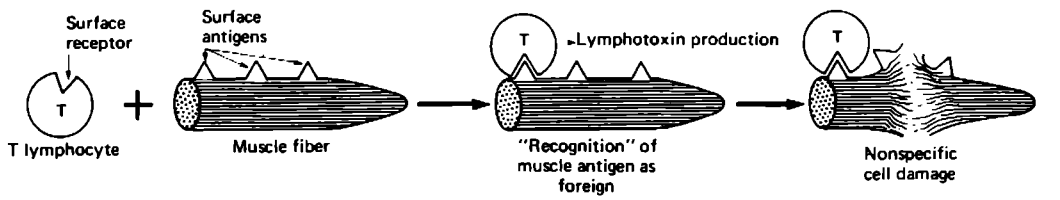

Figure 21–3. Defective "recognition" in polymyositis.

tients, consists of raised, smooth or scaling, dusky red plaques over bony prominences of the hands, elbows, knees, and ankles. An erythematous telangiectatic rash may appear over the face and sun-exposed areas. Less commonly seen is the pathognomonic "heliotrope" rash of the face (a dusky, lilac suffusion of the upper eyelids). One-fourth of patients have various dermatologic manifestations ranging from skin thickening to scaling eruptions to erythroderma.

4. Cancer–Some patients with polymyositis-dermatomyositis are found to have a concomitant malignant tumor. In men over 40 years of age, the association between polymyositis-dermatomyositis and cancer appears to be more common. Removal of the tumor may result in a dramatic improvement in the polymyositis-dermatomyositis.

5. Miscellaneous features–A mild transitory arthritis is not unusual. Sjögren's syndrome occurs in 5–7% of cases. In children, vasculitis may result in gastrointestinal ulceration with abdominal pain, hematemesis, and melena. Patients with severe muscle disease are particularly susceptible to the development of interstitial pneumonia and pulmonary fibrosis. Raynaud's phenomenon occurs occasionally.

B. Laboratory Findings: An elevated erythrocyte sedimentation rate and a mild anemia are very common. Half of patients have elevated α_2- and γ-globulins on serum protein electrophoresis. Myoglobinemia and myoglobinuria are often seen. Up to 20% of patients with acute polymyositis have nonspecific T wave abnormalities on the ECG.

1. Muscle enzymes–When muscle cells are injured, a number of muscle enzymes, including glutamic-oxaloacetic transaminase, creatine phosphokinase, and aldolase, are released into the blood. The serum enzyme elevation reflects the severity of muscle damage as well as the amount of muscle mass involved.

2. Urinary creatine–Creatine is normally produced in the liver and transported via the circulatory system to the musculature. After attaching to receptor sites on the muscle cell surface, it is carried into the cell, where it is converted to creatinine. Polymyositis-dermatomyositis and other myopathies lead to a decrease in the number of cell surface receptors, causing an increase in circulating creatine that is quickly cleared by the kidneys. An increase in the urine creatine concentration is the most sensitive laboratory test for muscle damage and is a valuable indicator of disease activity. It is the first detectable laboratory abnormality in relapse of disease.

3. Muscle biopsy–Biopsy of involved muscles is diagnostic in only 50–80% of cases. Therefore, a normal muscle biopsy does not rule out the diagnosis of polymyositis-dermatomyositis in a patient with a characteristic clinical picture, muscle enzyme elevations, and an abnormal electromyogram. The histologic findings in acute and subacute polymyositis-dermatomyositis include (1) focal or extensive primary degeneration of muscle fibers, (2) signs of muscle regeneration (fiber basophilia, central nuclei), (3) necrosis of muscle fibers, and (4) a focal or diffuse lymphocytic infiltration. Chronic myositis leads to a marked variation in the cross-sectional diameter of muscle fibers and a variable degree of interstitial fibrosis.

4. Electromyography–When involved muscles are examined, 70–80% of patients will demonstrate myopathic changes on electromyography. These changes are nonspecific but can point to the diagnosis of myositis. They include (1) spontaneous "sawtooth" fibrillatory potentials and irritability on insertion of the test needle; (2) complex polyphasic potentials, often of short duration and low amplitude; and (3) salvos of repetitive high-frequency action potentials (pseudomyotonia).

Immunologic Diagnosis

The diagnosis must be based on the nonimmunologic clinical and laboratory data discussed above. However, antibodies to the nuclear antigen Jo-1 are seen in a substantial number of patients with polymyositis, particularly those with pulmonary involvement. Antibodies to PM-Scl (formerly PM-1) are more common in patients with polymyositis and scleroderma. Anti-RNA antibodies occur most frequently in patients with myositis as a component of mixed connective tissue disease.

Differential Diagnosis

The 5 major criteria for the diagnosis of polymyositis-dermatomyositis are (1) weakness of the shoulder or pelvic girdle, (2) biopsy evidence of myositis, (3) elevation of muscle enzymes, (4) electromyographic findings of myopathy, and (5) typical skin changes. A number of diseases can affect muscles and lead to clinical and laboratory abnormalities that are identical to those seen in polymyositis-dermatomyositis. The diagnostic criteria outlined above cannot be strictly applied in patients with these diseases, which include infection, sarcoidosis, muscular dystrophy, SLE, progressive systemic sclerosis, mixed connective tissue disease, drug-induced myopathy (alcohol, clofibrate), rhabdomyolysis, and various metabolic and endocrine disorders (McArdle's syndrome, hyperthyroidism, myxedema, acid maltase deficiency, carnitine palmityl transferase deficiency, and AMP deaminase deficiency). A diligent search for occult cancers should be made in any patient who develops polymyositis-dermatomyositis as an adult.

Treatment

A. Corticosteroids: Prednisone, 60–80 mg orally daily, will usually decrease muscle inflammation and improve strength. The dose is tapered slowly, with clinical and laboratory monitoring. Creatinuria is the most sensitive index of disease activity and is often the first indication of relapse as corticosteroid dosage is reduced. However, assessment of muscle strength and determination of serum enzyme levels are usually sufficient indicators of disease activity. Some patients require chronic prednisone therapy (5–20 mg daily) to control the disease.

B. Cytotoxic Agents: Methotrexate or azathioprine has been used with success in patients who do not respond to corticosteroids or who develop severe complications of corticosteroid therapy.

Complications & Prognosis

Polymyositis-dermatomyositis is a chronic disease characterized by spontaneous remissions and exacerbations. Most patients respond to corticosteroid therapy. Patients with severe muscle atrophy show little response to either corticosteroid or other immunosuppressive therapy. When the disease is associated with cancer, the prognosis depends on the response to tumor therapy.

POLYARTERITIS NODOSA, WEGENER'S GRANULOMATOSIS, & OTHER VASCULITIDES

The vasculitides represent a spectrum of pathologic and clinical disease ranging from acute, overwhelming necrotizing vasculitis to chronic, indolent vascular inflammation. It is important to classify and to differentiate the vasculitides because the course, treatment, and prognosis are different for each disease. In this section we discuss similar disorders under a single heading. For example, polyarteritis nodosa of Kussmaul and Maier and allergic granulomatous angiitis are both discussed under the heading "polyarteritis nodosa."

Immunologic Pathogenesis & Diagnosis

No unifying pathogenetic mechanism has yet been defined for the vasculitides, but most are probably immunologically mediated disorders. Different inciting antigens could induce specific clinical responses or, alternatively, host factors (heredity) could determine the type of clinical response.

A. Humoral Pathogenesis:

1. Polyarteritis nodosa—Several animal experiments suggest that humoral factors may play a role in the pathogenesis of polyarteritis nodosa. Repeated intravenous injections of horse serum into rabbits will induce an arteritis similar to that seen in polyarteritis nodosa. Bovine albumin serially administered intravenously into rabbits will also induce a vasculitis. (Bovine γ-globulin administration induces immune complex renal disease with only minor inflammatory vascular changes.) The intravenous injection of immune complexes, particularly in the presence of rheumatoid factor, will induce vasculitis in rats. Immunofluorescence studies in humans have demonstrated immunoglobulin and complement in vessel walls during active disease. Hepatitis B antigen (HBsAg), either alone or with immunoglobulin and complement, has been demonstrated in the vessel walls of patients with polyarteritis nodosa who have circulating HBsAg (Fig 21–4). HBsAg or anti-HBsAg antibodies can be detected in the serum of over half of patients with polyarteritis nodosa.

2. Hypersensitivity angiitis—Vascular deposition of IgG, IgM, IgA, and complement in patients with hypersensitivity angiitis has been documented by immunofluorescence studies. In a specific type of hypersensitivity vasculitis known as Henoch-Schönlein purpura, examination of renal biopsies reveals granular and nodular deposition of IgA, IgG, and complement in the mesangium and along the glomerular basement membrane. Circulating IgA-containing immune complexes have been identified in two-thirds of patients, and skin biopsy studies have demonstrated IgA deposited in the walls of involved vessels.

3. Wegener's granulomatosis—Ultrastructural and immunofluorescence studies of renal biopsies from patients with Wegener's granulomatosis have demonstrated subepithelial basement membrane deposition of IgG and complement in a lumpy-bumpy pattern in glomerular tufts, characteristic of immune complex disease. Circulating autoantibodies against smooth muscle have been detected in a few patients, and circulating immune complexes have occasionally been detected by complement consumption techniques.

4. Takayasu's disease—Patients with Takayasu's disease have elevated serum IgG, IgA, and IgM levels. False-positive VDRL and rheumatoid factors are not uncommon. Circulating autoantibodies against vascular antigens have been demonstrated by sheep cell agglutination, complement fixation, and tanned red cell agglutination.

5. Giant cell arteritis—Immunofluorescence techniques occasionally reveal IgG, IgA, IgM, and complement deposition in the cytoplasm of vessel wall cells and along the elastic tissue within vessel walls. Antinuclear antibodies directed against nuclei of vessel wall cells have been detected in some patients.

B. Cellular Pathogenesis: Decreased delayed cutaneous hypersensitivity to various antigens (PPD, mumps, streptokinase-streptodornase, keyhole limpet hemocyanin), as well as decreased in vitro lymphocyte mitogenic responsiveness, has been documented in patients with **Wegener's granulomatosis.** However, most studies have been done on patients being treated with corticosteroids or cytotoxic drugs.

C. Antigens Associated With the Vasculitides:

1. Streptococcal antigens—Antecedent upper respiratory infections, occasionally streptococcal in origin, have been associated with Henoch-Schönlein purpura, hypersensitivity angiitis, and polyarteritis nodosa. A necrotizing arteritis can be induced in the coronary arteries of rats receiving repeated injections of hemolytic streptococcal antigens.

2. Viral antigens—Various viral infections (upper respiratory infections, influenza, influenza vaccination) are known to occasionally precede Henoch-Schönlein purpura, hypersensitivity angiitis, and polyarteritis nodosa. Hepatitis B infection may contribute to the development of polyarteritis nodosa (see above).

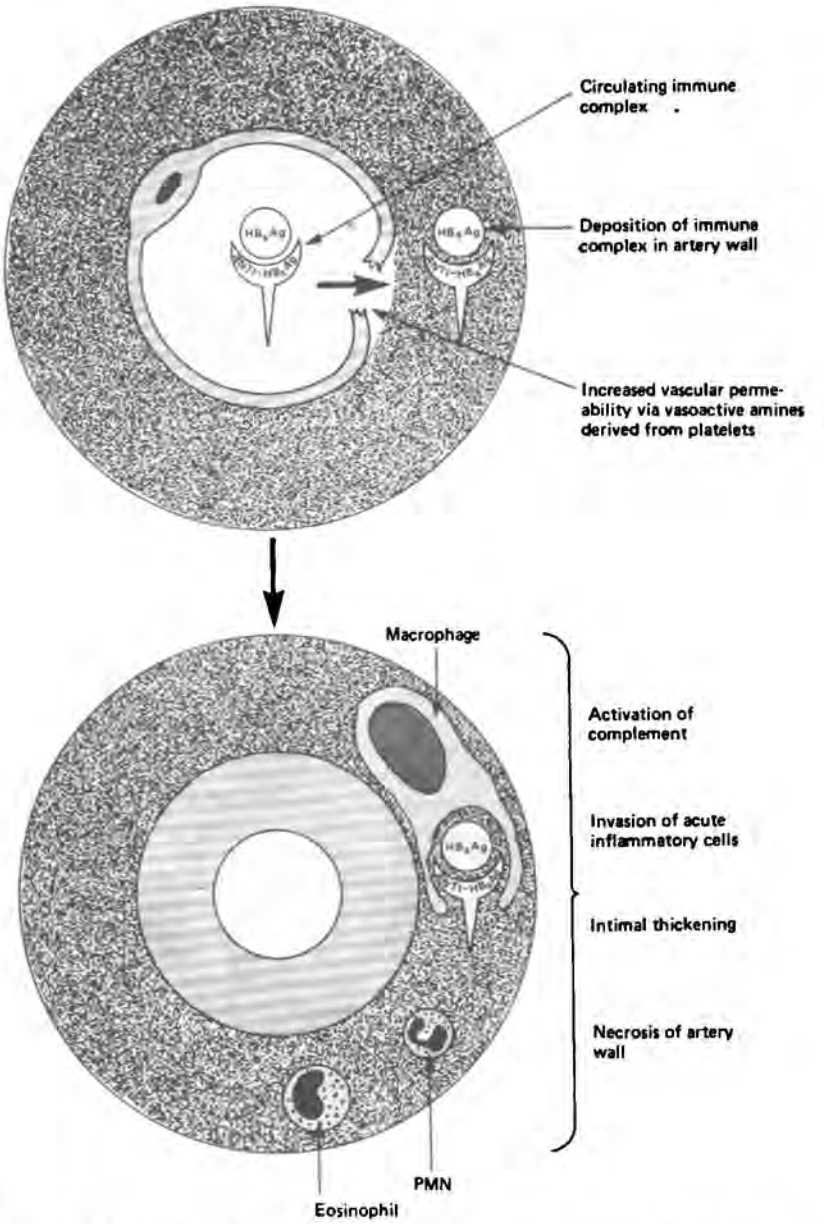

Figure 21–4. Hypothetical immunopathogens in HBsAg-associated polyarteritis nodosa.

3. Drugs–Drugs have been implicated as causes of polyarteritis nodosa, hypersensitivity angiitis, and Henoch-Schönlein purpura. Suspected agents include sulfonamides, penicillin, phenytoin, arsenicals, thiouracil, iodides, and thiazides. There is a very high incidence of polyarteritis nodosa in parenteral methamphetamine abusers, although there is controversy over the role of hepatitis B virus in these patients.

4. Autoantigens–Anti-vessel wall antibodies have been detected in some patients with Takayasu's disease. However, such antibodies could result from the vascular inflammatory process. Lymphocyte activation by homologous muscle has been demonstrated in patients with polymyalgia rheumatica.

Clinical Features

A. Symptoms and Signs:

1. Polyarteritis nodosa–Polyarteritis nodosa is a necrotizing vasculitis of small muscular arteries (0.5–1 mm in diameter). Involved vessels contain a

segmental perivascular inflammatory infiltrate that consists predominantly of PMNs and eosinophils. Segmental fibrinoid necrosis of the media and adventitia causes weakening of the vessel wall, and aneurysm formation occurs. As the lesion evolves, intimal proliferation results in luminal obliteration and ischemia of involved tissues. Finally, as inflammation subsides, the vessel is replaced by fibrous tissue. Biopsy of involved tissue from patients with classic polyarteritis nodosa may show lesions at all stages of development.

Polyarteritis nodosa usually occurs in men, who present with fever, malaise, and weight loss. The protean manifestations of polyarteritis nodosa reflect the multisystemic organ involvement of the disease.

a. Kidney–The kidneys are involved in 70% of patients, and renal insufficiency is the commonest cause of death. Renovascular hypertension develops in 50% of patients.

b. Heart–Sixty percent of patients have cardiac involvement, with congestive heart failure being the commonest manifestation. Myocardial infarction may be seen in up to 20% of patients.

c. Gastrointestinal tract–Half of patients develop vasculitis of the gastrointestinal tract, manifested by pancreatitis, hepatitis, cholecystitis, bowel infarction or perforation, and gastrointestinal bleeding.

d. Lungs–The lung may be involved pathologically in 25% of cases. Typical clinical manifestations include asthma, bronchitis, and pneumonia. Pulmonary infection complicates the course of half of patients with polyarteritis nodosa.

e. Muscles–Twenty percent of cases show signs of myositis such as pain, weakness, and tenderness.

f. Central nervous system–Eight to 25% of patients have some central nervous system abnormality. The major complication is infarction.

g. Skin–One-fourth of patients have a variety of skin manifestations, including livedo reticularis, subcutaneous nodules, and palpable purpura. In a small group of patients, skin disease may be the only manifestation of necrotizing vasculitis.

h. Testis–Although the testis is a favorite site for biopsy, only 19% of autopsy cases show testicular involvement. In the absence of signs or symptoms of testicular involvement, biopsy will probably be unproductive.

Polyarteritis nodosa was once thought to have a 5-year survival rate of only 10%. With better diagnosis and therapy, a number of investigators report 5-year survival rates of 80% or greater.

2. Hypersensitivity angiitis–The hypersensitivity angiitides are a group of disorders characterized by inflammation of arterioles and venules.

a. Primary hypersensitivity angiitis–Primary hypersensitivity angiitis occurs with equal frequency in both sexes, at any age, and often begins after an infection or drug treatment. Pathologically, hypersensitivity angiitis differs from classic polyarteritis nodosa in that smaller vessels (including capillaries) are involved and individual lesions appear to be at the same stage of development. The lungs, kidney, and skin are commonly involved, and gastrointestinal involvement is unusual. Hypertension is uncommon. Cases associated with a known antigen may go into remission when the offending agent is removed.

b. Henoch-Schönlein purpura (allergic purpura)–Henoch-Schönlein purpura usually occurs in boys. The histopathologic features by light microscopy are virtually identical to those of hypersensitivity angiitis, with involvement of venules and capillaries as well as small arterioles. Henoch-Schönlein purpura also frequently follows antigenic exposure, eg, infection or drugs, and some patients have a history of food allergies.

Patients with Henoch-Schönlein purpura demonstrate a fairly characteristic clinical picture, with nonthrombocytopenic purpura, colicky abdominal pain, arthralgias of the knees and ankles, and glomerulonephritis. Nephrotic syndrome and renal failure have been described, but these complications are more likely to occur in adults than in children. There is a high incidence of intestinal hemorrhage and intussusception. The disease is usually limited to a single episode lasting 4–6 weeks, but recurrences can occur upto 2 years thereafter.

c. Secondary hypersensitivity angiitides–Small-vessel vasculitis may occur in many pathogenetically different disorders, including connective tissue diseases, malignant disorders, hypergammaglobulinemia, cryoglobulinemia, bacterial endocarditis, primary biliary cirrhosis, and chronic inflammatory bowel disease.

3. Wegener's granulomatosis–Wegener's granulomatosis is a necrotizing granulomatous angiitis affecting arteries and veins. The 3 major areas of involvement are the upper respiratory tract, the lung parenchyma, and the kidneys. Men and women are affected with equal frequency. With involvement of the upper and lower respiratory tract, patients develop sinusitis, rhinitis, septal perforation, tracheobronchitis, asthma, and pneumonia. With the onset of renal involvement, the patient progresses rapidly to renal failure. Renal findings include necrotizing angiitis, focal glomerulitis with thrombosis of glomerular capillaries, crescent formation, glomerular and interstitial necrosis, and granuloma formation. As in hypersensitivity angiitis, hypertension is uncommon. Other manifestations of Wegener's granulomatosis include arthralgias, polyneuropathy, parotitis, prostatitis, pericarditis, myocarditis (occasionally with infarction), abdominal vasculitis, skin involvement, and ocular disease (exophthalmos, episcleritis, conjunctivitis, and corneal erosion).

Before modern methods of therapy became available, patients with Wegener's granulomatosis almost always died in renal failure, and even today a patient's renal status still determines the long-range outlook. Survival with current treatment regimens approaches 90%.

4. Takayasu's arteritis (pulseless disease, aortic arch syndrome, giant cell arteritis of the aorta)—Originally described in young women of Asian descent, Takayasu's arteritis is now known to occur throughout the world. It is an indolent inflammatory process involving the thoracic aorta and large arteries. The inflammation, which can be diffuse or focal, is characterized by mononuclear cell infiltration and giant cell formation in the media and adventitia of vessels large enough to have an elastic lamina. Degeneration of the media with disruption of the elastic layer leads occasionally to aneurysm formation. Proliferation of fibrous tissue may lead to narrowing and obliteration of the lumen of any involved vessel. Takayasu's syndrome is one of the only causes of stenosing aortitis. Extensive vascular calcification resembling that seen in atherosclerosis develops in patients with long-standing disease. Takayasu's disease has been described in patients with other rheumatic diseases such as SLE or rheumatoid arthritis.

Constitutional symptoms of fever, night sweats, and weight loss are common among patients with Takayasu's disease. Erythema nodosum and pyoderma gangrenosum may be seen. Carotid and vertebral arterial involvement leads to cerebrovascular insufficiency with transient ischemic attacks, vertigo, and intermittent visual disturbances. Takayasu's disease of the renal artery is associated with renovascular hypertension. Involvement of the femoral and iliac arteries leads to peripheral claudication. Either the coronary ostia or the coronary arteries themselves may become involved, leading to angina pectoris or infarction. The case fatality rate is 25–75% within 5 years, with a high incidence of sudden death.

5. Giant cell arteritis (temporal arteritis)—Giant cell arteritis is a disease of the elderly, being very unusual in patients under 55 years of age. Women are affected twice as often as men. Pathologically, the disease is characterized by nonsuppurative granulomatous inflammation (either focal or diffuse) of the aorta and large vessels. Giant cells are conspicuous on histologic examination.

Constitutional findings include fever, weight loss, and malaise. Patients characteristically complain of symmetric arthralgias, myalgias, morning stiffness, and weakness of pelvic and shoulder girdles. The characteristic morning headache, which is described as constant and "boring," may be limited to the temporal areas or may radiate widely over the head. The complications of the disease depend on the vessels involved. The temporal arteries may become warm, red, and tender, with or without painful nodules. With ocular vessel involvement, visual symptoms (ranging from intermittent blurring to diplopia to blindness) may result. Without treatment, blindness may occur in up to 30% of patients with acute giant cell arteritis. The manifestations of cerebrovascular involvement include transient ischemic attacks, stroke, and cranial nerve palsies. With involvement of the aorta and other large vessels, peripheral claudication or myocardial ischemia can be seen. Giant cell arteritis is usually a self-limited disease that lasts for approximately 2 years. In occasional patients, the disease may last for more than 5 years. Because of the high incidence of blindness, it is crucial to make the diagnosis and treat the disease. The definitive diagnostic technique is temporal artery biopsy, but a large segment of one or both temporal arteries is required because of the focal nature of the disease. The relationship of giant cell arteritis to polymyalgia rheumatica is a matter of some controversy. The 2 diseases have the same age and sex distribution, and the systemic symptoms are virtually identical. A large number of patients with polymyalgia rheumatica have giant cell arteritis on biopsy even without signs of temporal arteritis. At this time, polymyalgia rheumatica should be considered a clinical syndrome often, but not always, associated with giant cell arteritis.

B. Laboratory Findings: Many patients with vasculitis have leukocytosis (20,000–50,000/μL). Eosinophilia—often up to 1500/μL—occurs most commonly in patients with systemic necrotizing vasculitis with lung involvement. Anemia is very common and may be due to blood loss, microangiopathic damage, or chronic disease.

Proteinuria, hematuria, and granular or cellular casts may be seen with renal involvement. Azotemia is a common complication of the disseminated vasculitides. Nonspecific laboratory tests indicating inflammation, such as the erythrocyte sedimentation rate and the "acute-phase reactants" (eg, C-reactive protein), are almost universally abnormal. Polyclonal hypergammaglobulinemia is a common finding. Cryoglobulinemia, macroglobulinemia, antinuclear antibodies, and rheumatoid factors may all be seen.

C. X-Ray Findings: With active pulmonary involvement, the chest film may reveal multiple infiltrates with interstitial fibrosis or nodular densities with or without cavitation. Patients with Takayasu's disease may show diffuse vascular calcifications similar to those seen in atherosclerosis. On angiography, a high percentage of patients with polyarteritis nodosa, particularly those with abdominal or renal manifestations, will have microaneurysms in small arteries throughout the visceral circulation. Angiography may show beading and irregularity of involved arteries in patients with giant cell arteritis. Large-vessel stenosis and vascular calcification are the characteristic angiographic findings in patients with Takayasu's syndrome.

Treatment

A. Corticosteroids: Corticosteroids are useful in polyarteritis nodosa and hypersensitivity angiitis. They may result in rapid clinical improvement and have been shown to increase the 12-month survival rate in these 2 diseases. Carefully controlled studies of the effects of corticosteroids on long-term survival in these diseases have not been done. The suggested therapeutic regimen is prednisone, 60–80 mg orally daily, with clinical and laboratory measurements to determine the duration of therapy. Corticosteroids are also

the major mode of therapy in giant cell arteritis. In patients with polymyalgia rheumatica without temporal arteritis, the recommended treatment is prednisone in moderate doses, using the erythrocyte sedimentation rate and clinical symptoms to determine how fast to taper the drug. When temporal arteritis is suspected, a biopsy of the artery should be made immediately and the patient begun on prednisone, 60 mg orally daily, to forestall the possibility of blindness. These patients usually respond dramatically within 4 days. Corticosteroids should be tapered to the lowest dose required to control symptoms and therapy continued for 2 years, after which attempts should be made to withdraw corticosteroids completely. The use of corticosteroids in Takayasu's disease has been shown to control acute inflammatory reactions and probably to stop the progression of the disease. Prednisone is begun in a dosage of 30–60 mg orally daily and, after 9 weeks, is tapered to a maintenance dose of 5–10 mg daily. The duration and the long-term effectiveness of therapy have not been determined.

Corticosteroids are of little benefit in Henoch-Schönlein purpura. Although they decrease acute inflammatory symptoms and can be used when the response to aspirin is inadequate, they have no effect on the severity or progression of the renal disease.

B. Cytotoxic Agents: Cytotoxic drugs have dramatically improved the prognosis in Wegener's granulomatosis. Cyclophosphamide, starting at 1–2 mg/kg daily (orally or intravenously, depending on the severity of the clinical situation), has been employed most commonly. The drug is administered until there is a clinical response or until signs of toxicity develop (marrow suppression, gastrointestinal intolerance, alopecia, hemorrhagic cystitis). The duration of therapy required is unknown, but generally cyclophosphamide is administered for at least a year after the disease has resolved. Long-term remissions have been induced, so periodic attempts should be made to withdraw this potentially dangerous drug. Corticosteroids are used concurrently with severe inflammatory disease. Cytotoxic agents have also been shown to increase significantly the survival of patients with polyarteritis nodosa.

SERUM SICKNESS

Serum sickness is an adverse immunologic response to a foreign antigen, usually a heterologous protein. The incidence of the disease has declined with the decreasing therapeutic use of heterologous antisera; however, it still occurs following the administration of various heterologous antitoxins, including those for rabies, diphtheria, snake venom, and clostridia, and following administration of certain drugs (eg, penicillin, sulfonamides).

Signs and symptoms begin 7–15 days after exposure to the offending antigen and include fever, myalgias, arthralgias, arthritis, urticaria, lymphadenopathy, and splenomegaly. The arthritis may involve large or small joints, and although pain, swelling, and effusions are common, heat and erythema are seldom reported.

Laboratory evaluation reveals leukocytosis (occasionally with eosinophilia), hematuria, proteinuria, and decreased complement levels (CH_{50}). The synovial fluid white count is over 20,000/μL, mostly PMNs, and the complement level is decreased. The disease is usually self-limited, with no residua. Rare complications include laryngeal edema, mononeuritis, glomerulonephritis, and vasculitis.

Serum sickness is the prototype immune complex disease. During the initial immune response, there is antigen excess leading to the formation of soluble antigen-antibody complexes that diffuse into involved tissues, activate complement, and initiate the inflammatory response that causes the disease. As antibody titers rise and approach equivalence, insoluble complexes are formed that are quickly cleared by the reticuloendothelial system. Precipitating antibodies that fix complement can be demonstrated in most patients with serum sickness. Hemagglutinating antibodies against sheep red blood cells can be detected in virtually 100% of patients. Titers of hemagglutinating antibody rise following the onset of clinical manifestations and peak with clinical recovery.

The urticaria responds to epinephrine and antihistamine therapy, and salicylates are effective in controlling constitutional symptoms and the arthritis. A short course of corticosteroids may be required in severely ill patients.

BEHÇET'S DISEASE

Behçet's disease is a chronic recurrent inflammatory disease affecting adults of both sexes. The major manifestations of the disease are aphthous stomatitis, iritis, and genital ulcers. Other findings include vasculitis (particularly of the skin), arthritis, meningomyelitis, enterocolitis, erythema nodosum, thrombophlebitis, and epididymitis. The differential diagnosis includes viral (herpes simplex) or chlamydial (inclusion conjunctivitis, lymphogranuloma venereum) infections, Reiter's syndrome, inflammatory bowel disease, Stevens-Johnson syndrome, and SLE. A pustular lesion appearing after needle puncture of the skin is highly suggestive of Behçet's disease.

Genetic and environmental factors probably play a role in pathogenesis. Some studies show an increased prevalence of HLA-B5 in Behçet's disease, but there is also evidence suggesting that a virus may play a role in disease causation. Cerebrospinal fluid from patients with Behçet's disease will produce encephalitis, optic neuritis, uveitis, keratitis, and conjunctivitis in rabbits. A viral agent cultured from the eye, blood, and urine of one patient has produced encephalitis in mice. Antibodies against various human mucosal antigens have been detected, and indirect immunofluorescence has demonstrated vascular deposition of immunoglobulins as well as circulating anticytoplasmic antibodies

Recent studies have demonstrated a decrease in circulating helper T lymphocytes. Furthermore, lymphocytes and plasma cells are prominent in the perivascular infiltrate of Behçet's vasculitis. Amyloidosis may develop in these patients.

Local corticosteroids are useful in the treatment of mild ocular and oral disease. Systemic corticosteroids are helpful in the treatment of systemic manifestations, but chlorambucil is thought to be the most useful agent when dealing with ocular disease. Unproved remedies include whole blood transfusions, transfer factor, levamisole, colchicine, cyclosporine, and thalidomide.

ANKYLOSING SPONDYLITIS

Ankylosing spondylitis is a chronic progressive inflammatory disorder involving the sacroiliac joints, spine, and large peripheral joints. Ninety percent of cases occur in males, with the usual age at onset being the second or third decade of life.

The disease begins with the insidious onset of low back pain and stiffness, usually worse in the morning. Symptoms of the acute disease include pain and tenderness in the sacroiliac joints and spasm of the paravertebral muscles. Findings in advanced disease include ankylosis of the sacroiliac joints and spine, with loss of lumbar lordosis, marked dorsocervical kyphosis, and decreased chest expansion. Peripheral arthritis, particularly of axial joints, may be seen. Twenty-five percent of patients will also have iritis or iridocyclitis. Carditis with or without aortitis is seen in 10% of patients, with 1–4% progressing to insufficiency of the aortic valves. Rare complications include pericarditis and pulmonary fibrosis.

Patients with ankylosing spondylitis are seronegative for rheumatoid factor. Hypergammaglobulinemia and antinuclear antibodies are not seen in ankylosing spondylitis, but an elevated erythrocyte sedimentation rate and a mild anemia are common during active disease. Electrocardiographic abnormalities, such as atrioventricular block, left or right bundle branch block, and left ventricular hypertrophy reflect cardiac involvement. X-rays of the sacroiliac joints reveal osteoporosis and erosions early in the disease and sclerosis with fusion in advanced disease. Calcification of the anterior longitudinal ligament of the spine and squaring of the vertebrae are seen on lateral x-rays of the spine. Ossification of the outer margins of the intervertebral disk (syndesmophyte formation) may lead to fusion of the spine.

On pathologic examination, these patients have a chronic proliferative synovitis very similar to that of rheumatoid arthritis. The characteristic skeletal change in advanced disease is ossification of the sacroiliac joints and interspinous and capsular ligaments. Pathologic cardiac findings include focal inflammation and fibrous thickening of the aortic wall and the base of the valve cusps.

The physical findings in patients with severe osteoarthritis of the spine may resemble those of patients with end-stage ankylosing spondylitis. However, degenerative osteoarthritis begins much later in life, does not extensively involve the sacroiliac joints, and is characterized radiographically by osteophytes rather than syndesmophytes. The differentiation of ankylosing spondylitis from other diseases associated with sacroiliitis and spondylitis, such as psoriatic arthritis, Reiter's syndrome, regional enteritis, and ulcerative colitis, depends upon the presence or absence of the clinical and radiologic characteristics of those diseases.

The basic pathogenesis of ankylosing spondylitis is unknown. Although the presence of mononuclear cells in acutely involved tissue and the histologic similarity of the synovitis to rheumatoid arthritis suggest a possible immunologic mechanism, there are no real data to support an autoimmune pathogenetic mechanism. There is a strong genetic predisposition to ankylosing spondylitis. Several members of the same family are often involved, and twin concordance for ankylosing spondylitis has been described. Furthermore, 90% of patients with ankylosing spondylitis have HLA-B27. The gene that determines this specific cell surface antigen may be linked to other genes that determine pathologic autoimmune phenomena or that lead to an increased susceptibility to infectious or environmental agents. There is no specific immunologic diagnostic test.

The treatment of ankylosing spondylitis consists of giving anti-inflammatory agents to decrease acute inflammation and relieve pain and of instituting physical therapy to maintain muscle strength and flexibility. Therapy is designed to maintain a position of function even if ossification and ankylosis progress. Posturing exercises (lying flat for periods during the day, sleeping without a pillow, breathing exercises), the judicious use of local heat, and job modification are all part of a rational physical therapy program. Total hip replacement may offer considerable relief to patients with ankylosis of the hips, although recurrent ankylosis is sometimes a problem.

REITER'S SYNDROME

Reiter's syndrome is classically defined as a clinical triad consisting of arthritis, urethritis, and conjunctivitis. However, the arthritis is frequently accompanied by only one of the other characteristic manifestations. Although it usually affects men, it may also occur in women and children. The arthritis is recurrent or chronic, migratory, asymmetric, and polyarticular, involving primarily joints of the lower extremity. Fever, malaise, and weight loss occur commonly with acute arthritis. The urethritis is nonspecific and often asymptomatic. The conjunctivitis is mild, but 20–50% of patients develop iritis. Balanitis circinata, painless oral ulcerations, and keratoderma blennorrhagicum (thick keratotic lesions of the palms and soles) are mucocutaneous manifestations. Complications include spondylitis and carditis.

Most patients have a mild leukocytosis. The urethral discharge is purulent, and smear and culture are usually negative for *Neisseria gonorrhoeae*. Synovial fluid is sterile, with a white cell count of 2000–50,000/μL, mostly PMNs. The classic radiographic finding is fluffy periosteal proliferation of the heels, ankles, metatarsals, phalanges, knees, and elbows. Bony erosions may be seen in severe cases but rarely, if ever, occur in upper extremities.

Major diseases in the differential diagnosis include gonococcal arthritis, psoriatic arthritis, ankylosing spondylitis, and the arthritis of inflammatory bowel disease. Patients with psoriatic arthritis occasionally develop urethritis or conjunctivitis. The differentiation of psoriatic arthritis and Reiter's syndrome is difficult to make on the basis of the skin lesion, since keratoderma blennorrhagicum is histologically indistinguishable from pustular psoriasis. Reiter's syndrome can be differentiated from ankylosing spondylitis by the presence of the urethritis and conjunctivitis, the prominent involvement of distal joints, and the presence of asymmetric radiologic changes in the sacroiliac joints and spine.

The cause of Reiter's syndrome is not known. Some cases have been associated with sexual contact. Several infectious agents, including shigellae, salmonellae, gonococci, mycoplasmas, chlamydiae, yersiniae, and *Campylobacter* have been associated with Reiter's syndrome. Eighty percent of patients with Reiter's syndrome have HLA-B27. It is not known whether this antigenic marker imparts an increased susceptibility to environmental or infectious agents or is associated with an unusual immune response gene.

Salicylates, indomethacin, or one of the newer nonsteroidal agents may be used to control acute inflammation. Although the acute attack usually subsides in a few months, recurrences are common and some patients develop a chronic deforming arthritis.

PSORIATIC ARTHRITIS

Psoriatic arthritis is a chronic, recurrent, erosive polyarthritis seen in 5–7% of patients with psoriasis. The onset of the arthritis may be acute or insidious and is usually preceded by skin disease. It characteristically involves the distal interphalangeal joints of the fingers and toes and may involve the hips, sacroiliac joints, and spine. Distal interphalangeal joint disease is frequently accompanied by nail pitting or onycholysis secondary to psoriasis of the nail matrix or nail bed. Constitutional signs and symptoms, such as fever and fatigue, may occur. Severe erosive disease may lead to marked deformity of the hands and feet (arthritis mutilans), and marked vertebral involvement can result in ankylosis of the spine.

An elevated erythrocyte sedimentation rate and a mild anemia are common. Hyperuricemia is occasionally seen in patients with severe skin disease. Serum immunoglobulin levels are normal, and rheumatoid factor is absent. Synovial fluid examination reveals a white cell count of 5000–40,000/μL, mostly PMNs. Characteristic x-ray findings include "pencil cup" erosions, fluffy periosteal proliferation, and bony ankylosis of peripheral joints. Sacroiliac changes, including erosions, sclerosis, and ankylosis similar to that in Reiter's syndrome, occur in 10–30% of patients.

The major diseases that must be differentiated from psoriatic arthritis include rheumatoid arthritis, ankylosing spondylitis, and Reiter's syndrome. Psoriatic arthritis is differentiated from rheumatoid arthritis by the absence of rheumatoid factor and subcutaneous nodules, the involvement of distal interphalangeals, the characteristic x-ray findings of psoriatic arthritis, and the presence of psoriasis. The presence of the skin lesion, the involvement of distal interphalangeals, and differences in the radiologic appearance of the spine help differentiate psoriatic arthritis from ankylosing spondylitis. The differentiation of psoriatic arthritis from Reiter's syndrome is particularly difficult, since both diseases are associated with HLA-B27 and involve the sacroiliac joints and spine and since keratoderma blennorrhagicum is histologically indistinguishable from pustular psoriasis. A helpful clinical distinction is the greater likelihood of upper extremity involvement in psoriatic arthritis.

The cause of psoriasis and psoriatic arthritis is unknown. Genetic factors appear to play a role in disease causation. Psoriasis and rheumatic diseases are found in family members of 12–13% of patients. Twenty percent of patients with peripheral arthritis and 45% of patients with spondylitis have HLA-B27. Other HLA antigens may be associated with peripheral psoriatic arthritis. The high prevalence of genetic markers might be associated with an increased susceptibility to unknown infectious or environmental agents or to primary abnormal autoimmune phenomena. However, no immunologic pathogenetic mechanism has yet been demonstrated.

Skin and arthritic manifestations require therapy. Topical corticosteroids, coal tar and ultraviolet light, or immunosuppressive agents can be used to treat the skin disease. Treatment of arthritis is similar to that of rheumatoid arthritis.

RELAPSING POLYCHONDRITIS

Relapsing polychondritis is a rare disease characterized by recurrent episodes of inflammatory necrosis involving cartilaginous tissues of the ears, nose, upper respiratory tract, and peripheral joints. The disease often begins abruptly with swollen, painful, erythematous lesions of the nose or ears, usually associated with fever. The inflammation destroys supporting cartilaginous tissues, and patients are left with characteristic "floppy ear" and "saddle nose" deformities. Involvement of the upper respiratory tract leads to collapse of the trachea with recurrent bronchitis and pneumonia; the commonest cause of death in these patients is airway obstruction. Recurrent episcleritis, anterior

inflammatory ocular disease, auditory and vestibular defects, vasculitis, and arthritis are manifestations of relapsing polychondritis. Aortic insufficiency due to destruction and dilatation of the aortic valve ring occurs rarely.

Laboratory abnormalities include an elevated erythrocyte sedimentation rate, increased serum immunoglobulins, a false-positive VDRL, and mild anemia. Pathologic examination reveals infiltration of the cartilage-connective tissue interface with lymphocytes, plasma cells, and PMNs. As the lesion evolves, the cartilage loses its basophilic stippling and stains more acidophilic. Eventually, the cartilage becomes completely replaced by fibrous tissue.

The pathogenesis of this disease is unknown. However, there is some evidence that autoimmune phenomena play a role. Immunofluorescence has revealed the presence of immune complexes at the fibrocartilaginous junction. Antibodies to human cartilage and to type II collagen are present in a large number of patients. Electron microscopy reveals electron-dense deposits of lysosomal origin in involved cartilage. In some patients with relapsing polychondritis, cartilage antigen will induce lymphocyte activation and lymphocyte production of MIF. These observations must be verified and expanded before definitive statements on the immunopathogenesis of relapsing polychondritis can be made.

Corticosteroids, dapsone, colchicine, and nonsteroidal anti-inflammatory agents have been used with success in the treatment of relapsing polychondritis.

RELAPSING PANNICULITIS (Weber-Christian Disease)

Relapsing panniculitis is a rare syndrome characterized by recurrent episodes of discrete nodular inflammation and nonsuppurative necrosis of subcutaneous fat. Most patients are women. Painful, erythematous nodules usually appear over the lower extremities but may involve the face, trunk, and upper limbs and progress to local atrophy and fibrosis. Occasionally, they may undergo necrosis, with the discharge of a fatty fluid. Constitutional signs, including fever, usually accompany an acute episode. Histologically, one sees edema, mononuclear cell infiltration, fat necrosis, perivascular inflammatory cuffing, and endothelial proliferation. The differential diagnosis includes superficial thrombophlebitis, polyarteritis nodosa, necrotizing vasculitis, erythema induratum, erythema nodosum, and factitious disease.

The cause of relapsing panniculitis is not known, and in fact the syndrome may be simply a nonspecific response to any one of a number of inciting factors, including trauma, cold, exposure to toxic chemicals, and infection. It has been seen in patients with SLE, rheumatoid arthritis, diabetes mellitus, sarcoidosis, tuberculosis, withdrawal from corticosteroid therapy, acute and chronic pancreatitis, and pancreatic carcinoma. In some patients, it appears to be a hypersensitivity phenomenon, since it follows repeated injections of various drugs. An autoimmune mechanism is suggested by the association of relapsing panniculitis with several autoimmune diseases. In addition, patients with relapsing panniculitis may have hypocomplementemia or circulating immune complexes. However, the only autoantibodies demonstrated to date are circulating leukoagglutinins.

Acute episodes respond to corticosteroid therapy.

HEREDITARY COMPLEMENT DEFICIENCIES & COLLAGEN VASCULAR DISEASES

The past decade has seen the development of techniques for the evaluation and characterization of complement and complement inhibitors (see Chapter 10). The use of these techniques has revealed hereditary deficiencies of various components of the complement system. Complement deficiency is seen in one in a million normal adult males and is much more common in patients with various rheumatoid diseases.

Deficiencies of a number of complement components have been associated with several rheumatic disorders. In particular, deficiencies of C1r, C1s, C2, C4, C5, C6, C7, C8, and C1 esterase have all been associated with lupuslike syndromes. C2 deficiency is the most common hereditary complement deficiency.

The significance of hereditary complement deficiency in collagen vascular disease has yet to be clarified. Hereditary complement deficiency could lead to an increased susceptibility to infectious agents, particularly viruses, which may then stimulate the autoimmunity that results in disease. Alternatively, a neighboring gene predisposing to autoimmune phenomena could be inherited along with the defective gene for complement production.

At present there is no specific treatment for most complement deficiencies. Therapy is directed at the associated disease.

HYPOGAMMAGLOBULINEMIA & ARTHRITIS

Hypogammaglobulinemia is an acquired or congenital disorder that may involve all or any one of the specific classes of immunoglobulin (see Chapter 20). Hypogammaglobulinemia is associated with infections, chronic inflammatory bowel disease, sarcoidosis, SLE, scleroderma, Sjögren's syndrome, polymyositis-dermatomyositis, and malignancy. Patients with classic adult and juvenile rheumatoid arthritis may develop hypogammaglobulinemia.

Hypogammaglobulinemia patients may develop a seronegative, symmetric arthritis, with morning stiffness, occasional nodule formation, and radiographic evidence of demineralization and joint space narrowing. Bony erosions are rarely seen. Biopsy of the synovium reveals chronic inflammatory changes without

plasma cells. Despite the reduction of serum immunoglobulins, immunoglobulin may be detected in the inflammatory synovial fluid. Total hemolytic complement is commonly depressed in the synovial fluid, suggesting immune complex formation.

Hypogammaglobulinemia may result in an increased susceptibility to infection by unidentified viruses that may induce the autoimmune phenomena (including arthritis) in these patients.

Hypogammaglobulinemic arthritis may improve after the administration of gamma globulin.

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22 Hematologic Diseases

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There are few areas in hematology that are not significantly affected by immunologic processes. A large group of hematologic disorders—the plasma cell dyscrasias, lymphocytic leukemias, and lymphomas—represent abnormal proliferations of primary cells of the immune system. Another important group of disorders—the autoimmune hemolytic anemias, autoimmune neutropenias, and immune thrombocytopenias—are characterized by immunologic destruction of circulating blood cells. Even hematopoietic precursor cells in the bone marrow may be destroyed or suppressed by immunologic mechanisms, as seen in pure red cell aplasia and some cases of aplastic anemia.

This chapter will be devoted primarily to hematologic disorders in which immunologic cells or mechanisms play a major role. The chapter will also review other selected hematologic disorders in which immunologic observations have contributed significantly to the basic understanding or diagnosis of the disorder.

I. WHITE BLOOD CELL DISORDERS

Since many of the cells of the lymphoid system in peripheral blood and in tissues are included in the general category of white blood cells, it is not surprising that many diseases affecting these cells involve immunologic processes. These include the lymphoid malignancies—the plasma cell dyscrasias, leukemias, and lymphomas—and the nonmalignant disorders of infectious mononucleosis and some forms of leukopenia.

Traditionally, malignant cells in leukemias, lymphomas, and related disorders have been distinguished from their normal counterparts by morphologic, histochemical, and cytogenetic differences. Recently, immunologic techniques have been applied to cells in these disorders in an attempt to increase our understanding and improve our classification of these disorders. Immunologic classification has already shown clinical usefulness in acute lymphocytic leukemia, where prognosis and therapy depend partly on lymphocyte type. However, much additional basic and clinical work must be done before the full potential of immunologic classification of leukemias, lymphomas, and related disorders can be realized. Table 22–1 lists lymphoid disorders classified according to cell surface markers (see Chapter 18).

Table 22–1. Malignant diseases of the lymphoid system classified by cell surface markers.

T cell
Acute lymphocytic leukemia (20%)
Lymphoblastic lymphoma
Some other non-Hodgkin lymphomas
Sézary syndrome
Mycosis fungoides
Chronic lymphocytic leukemia (2%)
B cell
Chronic lymphocytic leukemia (98%)
Waldenström's macroglobulinemia
Multiple myeloma
Burkitt's lymphoma
Most other lymphocytic lymphomas
Hairy cell leukemia
Null cell
Acute lymphocytic leukemia (80%)
Some non-Hodgkin lymphomas
Histiocyte-monocyte
Acute monocytic leukemia
Malignant histiocytosis
Histiocytosis X
Hairy cell leukemia
Controversial
Hodgkin's disease

PLASMA CELL DYSCRASIAS

These diseases are also called the paraproteinemias or monoclonal gammopathies. They comprise a heterogeneous group of diseases characterized by the presence in serum or urine of a monoclonal immunoglobulin. This protein is also called a paraprotein, M protein (factor), or myeloma protein. It is the product of a single clone of lymphoid cells, is of restricted electrophoretic mobility, and appears in serum electrophoretograms as a narrow band or "spike" (Fig 22–1). The paraproteinemias are classified in Table 22–2.

SOURCE OF SPECIMEN	INTERPRETATION
Rheumatoid arthritis–serum	A broad polyclonal increase in γ-globulin
IgG-λ multiple myeloma–serum	A narrow, intensely staining monoclonal IgG-λ band in cathodal end of γ-globulin zone with little normal γ-globulin
IgG-κ benign monoclonal gammopathy–serum	A monoclonal IgG-κ band in anodal part of γ-globulin zone with normal γ-globulin staining
SLE–serum	A broad polyclonal increase in γ-globulin zone
IgM-κ Waldenström's macroglobulinemia–serum	An intense broad monoclonal IgM-κ band with very little migration of macroglobulin from application trough
Benign hypergammaglobulinemic purpura–serum	The monoclonal IgG-κ paraprotein forms a complex with normal IgG, producing a broad appearance midway between monoclonal and polyclonal
Normal human serum	Normal pattern

SOURCE OF SPECIMEN	INTERPRETATION
IgA-κ multiple myeloma–urine	Heavy proteinuria, IgA-κ paraprotein, κ Bence Jones L chains
IgA-κ multiple myeloma–serum	Monoclonal IgA aggregates in β_2 region
κ-Light chain myeloma–serum	Narrow β_1 peak consists of κ Bence Jones L chains
Chronic liver disease–serum	Very broad polyclonal increase in γ-globulin
Benign hypergammaglobulinemic purpura–serum	Monoclonal IgG-κ complexes with normal IgG to give broader appearance
IgG-κ multiple myeloma–serum	Narrow cathodal monoclonal IgK-κ band, very little normal γ-globulin
Normal human serum	Normal pattern

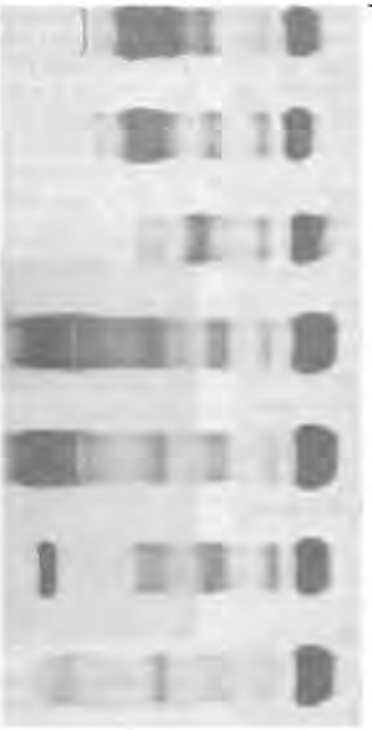

Figure 22–1. *Top:* Patterns of serum electrophoretograms from 7 subjects. They were run on agarose gel with the anode (on the right side) showing the albumin band. The heavy and light chain typing was determined by immunoelectrophoresis. *Bottom:* Patterns of serum and urine electrophoretograms run in agarose gel. The anode (albumin) is on the right. H and L chain types were determined by immunoelectrophoresis.

Table 22–2. Classification of plasma cell dyscrasias.

Malignant monoclonal gammopathy
Multiple myeloma
Waldenström's macroglobulinemia
Solitary plasmacytoma
Amyloidosis
Heavy chain diseases
Malignant lymphoma
Chronic lymphocytic leukemia
Light chain diseases
Secondary monoclonal gammopathy
Cancer (nonlymphoreticular)
Monocytic leukemia
Hepatobiliary disease
Rheumatoid disorders
Chronic inflammatory states
Cold agglutinin syndrome
Benign hyperglobulinemic purpura of Waldenström
Papular mucinosis
Immunodeficiency
Gaucher's disease
Benign monoclonal gammopathy
Transient
Persistent
Drugs

Immunologic Pathogenesis

Despite intensive study over many years, the actual causes of plasma cell dyscrasias remain unclear. The recent clarification of several areas of cell cooperation in regulation of immune responses has not yet included control of monoclonal B cell proliferation. In particular, the presumed antigen that triggers the initial B cell proliferation is generally unidentified in this group of disorders.

Another major unresolved problem is the time that elapses between the stimulus and the development of clinical disease. Recent research suggests that this is 2–3 years in multiple myeloma. However, some patients with a paraprotein have been followed for over 30 years with no clinical evidence of disease associated with their paraprotein. Attempts are presently under way to analyze the long-term behavior of these monoclonal tumors by using specific antigenic markers that are unique to a particular tumor in an individual patient. Thus, the idiotype of the paraprotein (see Chapter 4) is used as a tumor marker to study the distribution of lymphoid cells bearing that marker during the course of the disease.

Laboratory Investigation of Paraproteinemias

Serum paraproteins may be found (1) during routine screening of serum samples, (2) during investigation of a patient with an apparently unrelated complaint, and (3) when a patient has symptoms or signs suggesting malignant plasma cell dyscrasia. The laboratory investigations should include **hematologic tests,** including complete blood count with differential and reticulocyte count, erythrocyte sedimentation rate, and bone marrow examination; **routine clinical chemistry,** including serum levels of total protein, albumin, globulin, calcium, phosphate, electrolytes, alkaline phosphatase, uric acid, blood urea nitrogen, creatinine, and cholesterol; **hemostatic profile,** including bleeding and clotting tests, platelet count, and specific factor assays if indicated; **serum viscosity** measured in an Ostwald viscosimeter; **whole blood viscosity; radiologic examination,** including chest x-ray, skeletal bone survey, and CT scanning (useful in identifying paravertebral deposits); and **renal function tests,** including urinalysis, 24-hour protein, creatinine clearance, and measurement of renal acidification to rule out renal tubular acidosis.

The **immunologic tests** (see Chapter 17) that should be performed include serum protein electrophoresis and immunoelectrophoresis after separation of the serum at 37 °C to avoid loss of a serum paraprotein as a cryoprecipitate. Fig 22–1 demonstrates the various patterns of paraproteins and the inability to predict H chain class and L chain type from zone electrophoresis alone. The amount of paraprotein is measured by densitometric tracing (see Chapter 17). Immunologic typing of an IgG paraprotein by immunoelectrophoresis is demonstrated in Fig 22–2. This is routinely performed with antisera to detect γ, α, μ, κ, and λ chains. If no abnormality is shown with these antisera and other evidence suggests a malignant paraproteinemia, immunoelectrophoresis should be performed with antisera specific for δ and ϵ chains to detect rare cases with IgD or IgE myeloma proteins. The main feature in the immunoelectrophoretic pattern that identifies a paraprotein is the change in the shape of the precipitin arc. Other changes are the reduction in amount of normal immunoglobulin of the same class as the paraprotein and localized splitting of the precipitin lines. Almost all paraproteins show an ab-

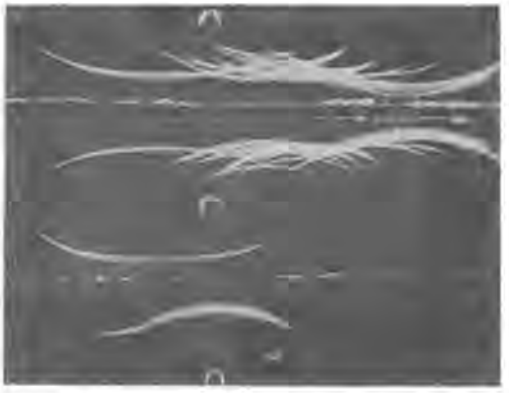

Figure 22–2. Immunoelectrophoresis of normal human serum (upper and center wells) and IgG myeloma serum (lower well). The upper trough contains antiserum to whole human serum and the lower trough antiserum specific to γ chains. Note the different shape and shorter precipitin line of myeloma IgG compared to normal IgG. (Reproduced, with permission, from Wells JV, Fudenberg HH: Paraproteinemia. *Disease-A-Month* [Feb] 1974.)

normal precipitin arc for either κ or λ chains that is similar in electrophoretic mobility to the H chain. The only exception is in H chain disease (see below).

All patients with paraproteins and suspected plasma cell dyscrasias without serum paraproteins must be tested for the presence of Bence Jones protein in the urine. Bence Jones protein consists of either monoclonal κ or λ light chains (see Chapter 4) that are excreted in significant amounts in about half of patients with multiple myeloma. They are best detected by zone electrophoresis and immunoelectrophoresis of the concentrated urine.

Immunofluorescence or immunoperoxidase microscopy on specimens of bone marrow with labeled specific antisera to various H and L chains can confirm the intracellular location of a monoclonal immunoglobulin in rare cases of nonsecretory myeloma (see below).

Ancillary investigations include tests for euglobulin (protein which precipitates at low ionic strength), cryoglobulin, rheumatoid factor, and cold agglutinins.

MULTIPLE MYELOMA

Major Immunologic Features

- Serum paraprotein (80%).
- Urinary paraprotein (50%).
- Reduced serum levels of nonmyeloma immunoglobulins.
- Recurrent infections.
- Presence of immature plasma cells in bone marrow.

General Considerations

Multiple myeloma is a disease characterized by the presence of a serum or urine paraprotein, anemia, and lytic bone lesions. It is the result of malignant transformation of a single clone of plasma cells. The diagnosis depends on the typical finding of large numbers of malignant plasma cells in the bone marrow, characteristic lytic bone lesions, and an associated serum or urine monoclonal protein.

Clinical Features

A. Symptoms and Signs: Bone pain and tenderness are common presenting features of multiple myeloma. Spontaneous pathologic fractures are not infrequent. Other major presenting features are anemia, recurrent infections (especially of the sinuses and respiratory tract), and occasionally renal failure or hypercalcemia.

B. Laboratory Findings: There is normocytic, normochromic anemia, with normal to slightly decreased white blood count and platelet count, and an elevated erythrocyte sedimentation rate. Uremia and hypercalcemia are common complications. The bone marrow shows increased numbers of plasma cells with many abnormal forms which often completely replace the marrow. The plasma cells may be arranged in sheets; many are large immature cells with large or multiple nuclei and nucleoli. Occasionally, plasma cells may be seen in peripheral blood.

C. X-Ray Findings: X-rays show characteristic punched-out lytic bone lesions throughout the skeleton in most patients. Generalized osteoporosis is also common. Rib and vertebral fractures are common.

Immunologic Diagnosis

Serum or urine paraproteins detected by zone electrophoresis (Fig 22–1) are typed by immunoelectrophoresis (see Chapter 17). Of all patients with multiple myeloma, about 50% have IgG paraproteins and 25% have IgA paraproteins. Serum levels of immunoglobulins other than the myeloma class are significantly lower than normal in almost all patients. Bence Jones protein is detected in the urine in about half of these patients. About 20% of patients have only Bence Jones proteinuria. These findings have important clinical and prognostic correlations.

IgD myeloma makes up 2% of cases of multiple myeloma but differs from the main group in several ways. Patients with IgD myeloma are generally younger than patients with other forms of multiple myeloma; over 50% have marked lymphadenopathy, hepatomegaly, or splenomegaly; and extraosseous lesions, amyloidosis, severe anemia, and uremia are more common. The serum levels of total protein and monoclonal protein are usually not very high, but Bence Jones proteinemia is common and Bence Jones proteinuria almost invariable. The L chain type is λ in 90% of cases of IgD myeloma compared with approximately 30% in IgG and IgA myelomas. The survival time in IgD myeloma is short—approximately 14 months after diagnosis.

In Bence Jones or L chain myeloma, the only detectable paraprotein occurs in the patient's urine as monoclonal κ or λ chains. This group makes up approximately 20% of myeloma patients. These patients are more likely to present with severe renal failure. The absence of an obvious serum paraprotein may present diagnostic difficulty in some cases, but Bence Jones proteinemia (monoclonal light chains in the serum) occurs in 80% and hypogammaglobulinemia and lytic bone lesions in over 60% of patients. Patients with λ type Bence Jones myeloma have more protein in the urine, poorer renal function, and shorter overall survival. Amyloidosis is more frequent in Bence Jones myeloma and may dominate the clinical course. Bence Jones myeloma has a poorer overall prognosis than IgG or IgA myeloma.

Nonsecretory myeloma refers to otherwise typical multiple myeloma with no paraprotein detectable in blood or urine; this accounts for approximately 1% of myeloma patients. The plasma cell tumor is incapable of secreting the synthesized intracellular monoclonal protein. This protein can be detected either by immunofluorescence microscopy of bone marrow or immunochemical analysis of a lysate of the tumor cells.

Some patients present with indolent disease and may not require therapy.

Differential Diagnosis

It is necessary to confirm by means of immunoelectrophoresis that the heavily stained band in the serum electrophoretogram is an immunoglobulin rather than fibrinogen, transferrin, or another protein.

Lytic bone lesions in an anemic patient may be metastases from a tumor of the breast, prostate, thyroid, kidney, or other primary site. The main distinguishing features are confirmation of a primary tumor or a history of its removal, the histopathologic features of the lytic lesion, abnormal plasma cells in bone marrow, and paraproteinemia. However, there are many reports of coexisting paraproteinemia and primary malignant nonlymphoid tumor.

Differentiation between clinically malignant and benign monoclonal gammopathies is discussed below with the latter group.

Treatment

A. General Measures: Supportive management is essential. Control pain with analgesics, provide adequate fluid intake, and give blood transfusions for symptomatic anemia. Ambulation should be maintained whenever possible to avoid further bone loss and hypercalcemia; adequate analgesics and mechanical support with corsets and braces often allow early ambulation, especially when the vertebral column is affected. Advice should be given regarding weight lifting and sports activities.

B. Irradiation: Local radiation therapy is very useful for relief of pain and reduction of tumor mass for isolated plasmacytoma or myeloma with localized painful bony lesions. Care must be taken to avoid large-scale radiation of active bone marrow sites, since this may cause severe pancytopenia and limit chemotherapy. Half-body irradiation generally precludes subsequent chemotherapy.

C. Chemotherapy: Cytotoxic chemotherapy, together with improved general management, will achieve good therapeutic responses in about 70% of patients with multiple myeloma and will produce increased median survival with improved quality of life. Response is gauged by clinical and laboratory observations such as general status and pain relief, hemoglobin concentration, serum or urine paraprotein levels, and plasma cell counts in the bone marrow.

Melphalan with or without prednisone is usually the drug regimen of choice for the initial treatment of myeloma. The most popular schedule for the use of melphalan is intermittent high-dose melphalan-prednisone for 4 days every 4–6 weeks. Melphalan is a stem-cell toxin, and the white cell and platelet count must be checked frequently to avoid toxicity. Poor-risk patients, ie, patients who present with renal failure, serious infection, hypercalcemia, leukopenia, thrombocytopenia, extensive lytic bone disease, and very large amounts of M protein, have a significantly shorter mean survival than patients who do not have these abnormalities.

Cyclophosphamide is also a widely used alkylating agent for the treatment of myeloma. It is probably as effective as melphalan but has additional adverse side effects. It is sometimes useful for patients who have become refractory to melphalan. It does have the advantage of being less toxic to bone marrow stem cells.

Multiple drug regimens are currently being assessed in patients who fail to respond to a single alkylating agent initially or who respond and then relapse. Combination chemotherapy may also be used for initial therapy for patients with high-risk disease (see above). The drugs used include melphalan, cyclophosphamide, carmustine (BCNU), procarbazine, vincristine, doxorubicin, and other agents in various combinations. The recently used combination of vincristine and adriamycin in 4-day infusions with oral dexamethasone (VAD) is promising in some patients in relapse. Occasional favorable responses result. Sequential half-body irradiation may have a part to play in some patients.

D. Surgery: Emergency laminectomy followed by local radiation therapy is indicated for acute spinal cord compression. Internal fixation of long bone fractures and prosthetic hip replacement may allow early return to ambulation after pathologic fractures. Occasionally, surgical cure of a solitary plasmacytoma may be successful.

E. Complications:

1. Infections—Recurrent bacterial infections, especially with pneumococci and sometimes with gram-negative organisms, are a major problem in some patients and are the most common cause of death. Timely evaluation of fevers and other symptoms of infection, with appropriate cultures and prompt coverage with appropriate antibiotics, is essential in these patients, especially during initial therapy. Gamma globulin or even, rarely, fresh-frozen plasma may also be useful in the treatment of established bacterial infection. Prophylactic administration of antibiotics, γ-globulin, or both is not warranted as a routine measure.

2. Hypercalcemia—This may present as an emergency with vomiting, dehydration, uremia, coma, and cardiac arrhythmias. Rapid rehydration is essential. Patients usually respond to rehydration, saline diuresis, and high doses of corticosteroids; refractory cases may require phosphate infusion or mithramycin.

3. Renal failure—Both acute and chronic renal failure are common. Etiologic factors include preexisting disease, precipitation of the paraprotein in tubules, amyloidosis, hypercalcemia, hyperuricemia, invasion of the kidney by malignant plasma cells, precipitation of cryoimmunoglobulins, hyperviscosity syndrome, pyelonephritis, and nephrotoxic antibiotics. Renal disease appears more frequently and often is more severe in patients with light chain myeloma (Bence Jones paraproteins). Light chains are toxic to renal tubular cells; precipitation occurs at acid pH levels. Prevention of dehydration is important in these patients. Fluid restriction should be avoided, especially fluid restriction for intravenous urography. Rapid treatment of complications such as hypercalcemia and hyperuricemia will help to avoid irreversible renal

damage. Successful chemotherapy of multiple myeloma will prevent most of the factors leading to renal failure. Hemodialysis or plasma exchange (or both) may be lifesaving and allow time for chemotherapy in patients with acute renal failure due to Bence Jones paraproteinemia.

4. Spinal cord compression—Acute spinal cord compression is fortunately an uncommon initial presentation of myeloma but requires emergency laminectomy when it occurs. Treatment for cord compression of gradual onset includes localized radiotherapy, chemotherapy, and supportive physiotherapy, including a brace. Despite the frequent radiologic evidence of widespread involvement of the vertebral column and frequent vertebral compression fractures, cord compression is not a frequent complication. Continued ambulation with adequate analgesia must be maintained if at all possible. Advice should be given about lifting heavy weights.

5. Hyperviscosity syndrome—This occurs occasionally in multiple myeloma and is discussed below with Waldenström's macroglobulinemia.

6. Acute leukemia—Many cases of acute leukemia have now been reported developing 1–10 years after a diagnosis of malignant paraproteinemia has been made, especially multiple myeloma. Almost invariably the leukemia is of the acute monocytic or myelomonocytic type. This is a serious complication; there are no reports of survivals beyond 6 months after diagnosis. It is not yet known if the leukemia is part of the natural history of multiple myeloma that only becomes obvious with prolonged survival or if it is due to chromosomal or other abnormalities induced by cytotoxic therapy. Dyserythropoietic anemia and chromosomal abnormalities may sometimes be detected prior to overt leukemia.

Plasma cell leukemia is a variant of multiple myeloma where abnormal plasma cells are found not only in the bone marrow but also in the peripheral blood. Plasma cell leukemia may be acute or chronic. Acute plasma cell leukemia presents as acute leukemia, and a mistaken diagnosis of acute lymphocytic or acute myelogenous leukemia may be made. The true nature of the leukemic cell is recognized when a paraprotein is detected in blood or urine, lytic bone lesions develop, severe hypercalcemia is noted, or renal failure supervenes. Plasma cell leukemia also occurs in patients with known multiple myeloma; such patients have a much higher incidence of hepatosplenomegaly, more severe anemia and thrombocytopenia, more renal failure, and a poorer prognosis than myeloma patients without plasma cell leukemia. Occasionally, plasma cell leukemia occurs as a terminal event in multiple myeloma, with a rapidly rising plasma cell count and death from infection and renal failure.

Therapy of plasma cell leukemia should be directed at the underlying multiple myeloma, although cell cycle-specific drugs, similar to those used in other acute leukemias, may be more useful than alkylating agents. The overall prognosis of plasma cell leukemia is poor.

Prognosis

Seventy percent of patients with multiple myeloma respond to therapy and have a mean overall survival of more than 30 months. Patients presenting with serious infection, thrombocytopenia, leukopenia, hypercalcemia, or irreversible uremia have a poorer prognosis. A large amount of paraprotein and a low serum albumin indicate a large plasma cell tumor mass and are associated with a poorer prognosis. Patients with IgG-type myeloma proteins tend to do better than those with IgA-type or Bence Jones proteins. The rate of response to chemotherapy also appears to be useful in predicting prognosis. Patients who do not respond to chemotherapy at all and those who respond very rapidly to initial chemotherapy have a poorer prognosis. The "fast responders" quickly relapse and often are resistant to further chemotherapy. The prognosis appears to be best for the "slow responders," ie, patients who show a slow but gradual and steady response to chemotherapy.

Radiologically proved bone healing occurs in approximately 30% of patients who respond to treatment, generally after a fall in serum paraprotein concentration. Serial observations of lytic lesions in the skull, ribs, and pelvis provide an index of disease activity in long-term management. However, bone healing is not a prognostic sign, since patients who respond to therapy and show bone healing do not have longer remissions or longer overall survival times than those who respond without bone healing.

WALDENSTRÖM'S MACROGLOBULINEMIA

The main clinical and laboratory features of Waldenström's macroglobulinemia are listed in Table 22–3 and compared to those of multiple myeloma. Most of the clinical manifestations of this disease can be directly attributed to the excess monoclonal IgM in the blood. Patients frequently present with hypervis-

Table 22–3. Comparison of clinical and laboratory features of Waldenström's macroglobulinemia and multiple myeloma.

	Macroglobulinemia	Multiple Myeloma
Recurrent bacterial infections		+++
Bone pain		+++
Lytic bone lesions		+++
Bleeding from mucosal areas	+++	+
Hepatosplenomegaly	+++	+
Lymphadenopathy	+++	+
Neuropathy		+
Changes in visual state	+++	+
Abnormalities in optic fundus	+++	+
Anemia	++	+++
Leukopenia		-
Thrombocytopenia		-
Hypercalcemia		++
Serum hyperviscosity	+++	-
Renal insufficiency		++

Table 22–4. Symptoms and signs of hyperviscosity syndrome.

System	Findings
General	Weakness, fatigue, malaise, anorexia.
Cardiovascular	Congestive heart failure, hypervolemia.
Neurologic	Headache, dizziness, vertigo, nystagmus, deafness, somnolence, stupor, coma, generalized seizures, electroencephalographic abnormalities.
Hematologic	Recurrent epistaxis, bleeding from oral mucosa, hematuria, hematemesis, melena, prolonged postoperative bleeding, anemia.
Ocular	Loss of visual acuity (may be total), retinal hemorrhages, distention and tortuosity of retinal veins, papilledema.

cosity syndrome (Table 22–4). A relative serum viscosity of greater than 3.0 may be associated with symptoms, although severe symptoms do not usually occur until the viscosity is greater than 7.0–10.0 (see Chapter 17). Increased whole-blood viscosity is also a feature. Severe hyperviscosity is a medical emergency and requires prompt hydration and plasma exchange. There is marked variation in the level of viscosity that causes symptoms in patients, but each patient tends to develop the same symptoms at the same viscosity level. Several factors contribute to hyperviscosity, including the serum concentration of monoclonal IgM, polymer or aggregate formation, cryoprecipitation, antibody activity against serum proteins, and red cell and vascular factors, including associated disorders of red cells.

Monoclonal IgM mainly exists in the pentameric 19S form or aggregates thereof, but many patients also have monomeric 7S IgM. A biosynthetic abnormality often exists in which the malignant clone is not always able to assemble all of the synthesized IgM in the pentamer form. Bence Jones proteinuria is found in approximately 10% of patients.

Plasma exchange is the treatment of choice for most patients with Waldenström's macroglobulinemia to remove the excess IgM and restore the plasma volume to normal, using a cell separator. Subsequently, plasma exchange is performed on a maintenance basis to keep the patient free of hyperviscosity symptoms until chemotherapy is effective. Chlorambucil is usually given in low doses on a daily basis, with frequent monitoring of blood counts to prevent bone marrow depression. Intermittent high-dose chlorambucil and prednisone—or cyclophosphamide, vincristine, and prednisone—can be used if there is no response to low-dose chlorambucil.

SOLITARY PLASMACYTOMA

Solitary plasmacytoma is an isolated malignant plasma cell tumor that can occur as a solitary plasmacytoma of bone or an extramedullary plasmacytoma. The patient has no other clinical or bone marrow features of multiple myeloma. Solitary plasmacytoma may be discovered on routine x-ray or may be diagnosed in a patient who complains of local pain or pressure on adjacent structures, eg, the spinal cord. In one study, 63% of patients with solitary plasmacytomas had a circulating or urinary monoclonal protein. Solitary plasmacytoma of bone and extramedullary plasmacytoma probably represent different diseases. Extramedullary plasmacytoma often has an indolent course, is commonly nonsecretory, and occasionally progresses to multiple myeloma. Solitary plasmacytoma of bone has a higher incidence of paraproteins, frequently progresses to multiple myeloma, has a poorer prognosis, and may represent an early form of multiple myeloma.

Solitary plasmacytoma is generally treated by excision (depending on the site) or high-dose local radiotherapy. The role of chemotherapy is disputed. Despite treatment, a significant number of patients will develop local recurrence or generalized multiple myeloma. Close follow-up and regular evaluation of serum and urine protein is essential, since multiple myeloma may develop many years after the diagnosis of solitary plasmacytoma.

AMYLOIDOSIS

Amyloidosis has proved an enigma to clinicians and pathologists ever since the 19th century, when Rokitansky and Virchow argued about its nature and origin. Its relationship to chronic infection and prolonged antigenic stimulation is well known. Amyloid has a complex structure, and several distinct components can produce amyloid fibrils with comparable biophysical properties. Solubilization of amyloid fibrils and amino acid sequence analysis show that in most cases a major component of amyloid is a fragment of an immunoglobulin L chain, especially the V region. This explains the negative results of earlier tests with antisera to detect immunoglobulins in amyloid tissue, since such antisera are made against C region and not V region determinants. Antiserum prepared against V region determinants of a Bence Jones protein from a patient with Bence Jones proteinuria and amyloidosis reacted with a component in that patient's amyloid tissue. Identical amino acid sequences in a patient's Bence Jones protein V region and a peptide component of his amyloid tissue have also been described.

Possible mechanisms that might account for the deposition of immunoglobulin components in amyloid tissue are as follows: (1) Catabolism by macrophages of deposited antigen-antibody complexes. (2) De novo synthesis in situ of whole immunoglobulins or of L chains with reduced solubility. (3) Genetic deletions in the L chain gene, producing an anomalous protein of reduced solubility. (4) Separate synthesis of discrete regions of the L chain.

The major nonimmunoglobulin component of amyloid is known as nonimmunoglobulin protein of unknown origin, amyloid of unknown origin, or protein "A." It has a molecular weight of approxi-

mately 8000 (76 amino acids), and it may be derived by proteolytic digestion of an unidentified protein precursor. Approximately 10% of amyloid tissue consists of doughnutlike structures 8–10 nm in diameter composed of 5 globular subunits surrounding a central cavity. The "P," or plasma, component may aggregate into "periodic rods" with a periodicity of 4 nm. It is a glycoprotein, unrelated to fibril protein but related antigenically to an α_1-globulin present in small amounts in normal human plasma. This protein may be measured by radioimmunoassay.

Clinically suspected amyloidosis must be confirmed by biopsy of appropriate tissues. Light microscopy of H&E-stained sections shows amyloid as an eosinophilic material. Typical birefringence occurs when stained sections are examined under polarized light. Electron microscopy shows characteristic nonbranching fibrils 8.5 nm wide and of varying lengths.

The classification of amyloidosis as primary or secondary is of little benefit etiologically or clinically. Amyloid fibrils of L chain origin can occur in both primary and secondary forms, as can the nonimmunoglobulin protein of unknown origin. The Third International Symposium on Amyloidosis recommended adoption of a system of nomenclature for the chemical composition of amyloid of different types, and this is incorporated into Table 22–5. The first of the 2 letters ("A") denotes amyloid fibril protein. The second letter indicates the nature of the protein, the tissue or organ, or the disorder in which it is found. Thus, AA indicates the main nonimmunoglobulin component (and SAA its serum-related protein); AL, the L chain amyloid protein; AS, senile amyloid; etc.

The clinical features reflect the particular site and extent of amyloid deposition, especially in the gastrointestinal tract, nervous system, and kidneys. Renal or cardiac involvement is often a poor prognostic sign. Treatment is mainly symptomatic, since no chemotherapy has been shown to consistently benefit patients with established amyloidosis. Patients with amyloidosis and plasma cell dyscrasia occasionally respond to melphalan or other drugs; rarely, full remission may be achieved.

HEAVY CHAIN DISEASES

These relatively rare diseases are characterized by a serum paraprotein composed of incomplete H chains without L chains. The 3 types are γ, μ, and α, with α chain disease the most prevalent. The paraprotein is generally in relatively low concentration in blood and shows broad zone electrophoretic pattern, but it is frequently excreted in urine in measurable amounts. Immunoelectrophoresis confirms the diagnosis, with demonstration of a paraprotein that reacts with antiserum to γ, μ, or α chains but not to κ or λ chains. No cases of δ or ϵ disease have been reported.

Much information on the genetic aspects of immunoglobulin biosynthesis has come from analysis of the paraproteins in H chain diseases. The abnormalities include partial deletion in the Fd portion of the H chain (with a normal amino acid sequence from residue 216), deletion in the hinge region (see Chapter 4), or a combination of the 2 findings.

Clinical Features

A. Gamma Chain Disease: The clinical features in γ chain disease vary markedly—from a malignant process with death within weeks of presentation to a course extending over 20 years. The most common presentation is a lymphoproliferative disorder with hepatosplenomegaly, lymphadenopathy, and uvular and palatal edema. Recurrent febrile episodes are not uncommon. Infection is the most common cause of death. The peripheral blood generally shows anemia, leukopenia, and atypical lymphocytes or plasma cells. Remission was obtained in one such patient treated with intermittent cyclophosphamide and prednisone.

B. Alpha Chain Disease: The clinical features in α chain disease are those of a severe malabsorption syndrome with chronic diarrhea, steatorrhea, weight loss, hypocalcemia, and lymphadenopathy. Biopsy of bowel shows infiltration of the small bowel with plasma cells, lymphocytes, and reticulum cells. Initially, the plasma cell infiltration of the lamina propria and mesenteric lymph nodes appears benign, suggest-

Table 22–5. Classification of amyloidosis.

	Clinical Type	Sites of Deposition	Chemical Type of Fibril*
Familial	Amyloid polyneuropathy (Portuguese, dominant inheritance)	Peripheral nerves, viscera	AF_{μ} (prealbumin)
	Familial Mediterranean fever (recessive)	Liver, spleen, kidneys, adrenals	AA
Generalized	Primary	Tongue, heart, gut, skeletal and smooth muscles, nerves, skin, ligaments	AL
	Associated with plasma cell dyscrasia	Liver, spleen, kidneys, adrenals	AL
	Secondary (infection, inflammation)	Any site	AA
Localized	Lichen amyloidosis	Skin	AD
	Endocrine-related (eg, thyroid carcinoma)	Endocrine organ (thyroid)	AE (AE_1)
Senile		Heart, brain	AS_c AS_b

*See text for explanation of abbreviations in this column.

ing an early premalignant phase. Patients at this stage may show regression of their abnormalities with oral antibiotic therapy. With disease progression, the plasma cells become more immature and extend beyond the lamina propria. Abdominal lymphoma and α chain disease are closely associated in the area of the Mediterranean Sea, and a recent study confirmed that they share identical etiologic, clinical, pathologic, and immunologic features. The diseases also occur in many more geographical areas. Two reported cases of α chain disease involved the respiratory tract instead of the gastrointestinal tract. Improved diagnosis of α chain disease is now achieved by immunodiffusion techniques with an antiserum specific for the Fab fragment.

C. Mu Chain Disease: The clinical features of μ chain disease are those of long-standing chronic lymphocytic leukemia with progressive hepatosplenomegaly.

BENIGN MONOCLONAL GAMMOPATHY

Benign monoclonal gammopathy is defined as the presence of a monoclonal serum or urine protein without any of the other manifestations of malignant plasma cell dyscrasia. Monoclonal protein spikes have been found in approximately 5% of all persons over 50 years of age and 8% of those over 70 years. Some of these people will ultimately develop multiple myeloma, but most will not.

Clinically, the problem is deciding whether a patient found to have a small monoclonal immunoglobulin spike has early multiple myeloma or benign monoclonal gammopathy. The following laboratory features tend to support a diagnosis of malignant paraproteinemia: serum paraprotein level greater than 2 g/dL, reduced serum levels of nonmonoclonal immunoglobulins, presence of immunoglobulin fragments in serum, increased serum or urine light chains, presence of radiographic bone lesions, presence of increased and abnormal plasma cells in the bone marrow, and, most importantly, increasing serum or urine paraprotein levels with time. Thus, the patient with a high and increasing serum paraprotein level, low serum levels of normal immunoglobulins, and significant amounts of Bence Jones protein in the serum and urine is likely to develop the full clinical picture of multiple myeloma within a relatively short period of time. Decreased total numbers of circulating B lymphocytes are present in malignant but not benign monoclonal gammopathies.

It is essential to review all patients with monoclonal gammopathies until the benign or malignant nature of the disease is established. Even in patients with proved benign monoclonal gammopathy, follow-up is required for a prolonged period, since multiple myeloma may supervene after an interval of as long as 24 years.

CRYOGLOBULINEMIA

A number of serum and plasma proteins precipitate at low temperature, including cryofibrinogen, C-reactive protein-albumin complex, heparin-precipitable protein, and immunoglobulins. The first step in evaluating a suspected cryoimmunoglobulin, therefore, is to rule out nonimmunoglobulin cryoproteins (see Chapter 17). The following points should be kept in mind when testing for a cryoglobulin: (1) Some monoclonal cryoglobulins may precipitate at temperatures as high as 35 °C. Adequate precautions such as prewarming of syringes, containers, etc, and centrifugation at 37 °C should therefore be taken to avoid loss of the cryoprotein from the supernate on centrifugation. (2) Some cryoglobulins rapidly precipitate in the cold, while others may take days. The serum should therefore be observed at 4 °C for at least 72 hours. (3) Most normal people have a small amount of polyclonal serum cryoglobulin—up to 80 μg/mL.

Cryoglobulinemias can be classified into the following 3 immunologic and clinical types:

Type I (25%) cryoglobulins are monoclonal proteins, most commonly IgM, occasionally IgG, and rarely IgA or Bence Jones protein.

Type II (25%) are mixed cryoglobulins with a monoclonal component. The monoclonal protein is usually IgM but is occasionally IgG or IgA, and it complexes with autologous normal IgG in the cryoprecipitate.

Type III (50%) are mixed polyclonal cryoglobulins, with a mixture of polyclonal IgM and IgG being by far the most frequent combination.

The clinical features in patients with cryoglobulinemias depend largely on the type of cryoglobulin involved. Patients with monoclonal cryoglobulins (type I) suffer primarily from the symptoms of their underlying disease process, eg, multiple myeloma or Waldenström's macroglobulinemia. Patients with "mixed cryoglobulins" (types II and III) often have "immune complex disease," with vascular purpura, arthritis, and nephritis. These immune complexes often fix complement in vivo and in vitro.

Treatment of cryoglobulinemias is generally directed toward treatment of the underlying disorder if it is recognized. General measures such as avoidance of cold objects or weather are often helpful. Management of the idiopathic mixed cryoglobulinemias may be difficult. Cytotoxic drugs, with or without prednisone, are generally used. Excellent remissions with reduction of serum cryoglobulin levels and control of nephritis, arthritis, and purpura can sometimes be achieved. Plasma exchange may be useful for short-term control of serious complications.

BENIGN HYPERGAMMAGLOBULINEMIC PURPURA

This relatively rare disease was described by Waldenström and occurs especially in young and mid-

dle-aged women who present with a dependent purpuric rash precipitated by exercise or alcohol. Some of these women have autoimmune disorders, especially SLE and Sjögren's syndrome.

The characteristic immunologic finding is a monoclonal IgG-κ paraprotein that acts as a rheumatoid factor and complexes autologous normal IgG to produce a broad appearance in the γ region on electrophoresis (Fig 22–1). Serum levels of IgA and IgM are normal or increased. There are no findings to support a diagnosis of multiple myeloma.

Treatment is directed mainly at correction of any underlying autoimmune disorder and avoidance of factors that obviously exacerbate the purpuric rash, such as excessive alcohol consumption and dancing. Rarely, the symptoms may be severe enough to warrant more active treatment such as plasma exchange.

BICLONAL GAMMOPATHY

More patients are being recognized whose serum contains 2 distinct paraproteins. This does not include the combination of a paraprotein and its corresponding Bence Jones protein. The most common combinations are 2 different monoclonal IgM proteins or monoclonal IgM and IgG proteins. Rarely, one may find 3 monoclonal serum proteins in one patient. Some of these patients are of great interest because their paraproteins show sharing of identical parts of their primary structure, and this fact supports the **genetic switch hypothesis.**

The clinical features are most frequently those of macroglobulinemia or lymphoma, and IgM is generally the monoclonal protein in highest concentration. Plasma exchange is frequently required in these patients.

LEUKEMIAS

The leukemias are characterized by abnormal maturation and accumulation of white blood cells. They are classified as acute or chronic on the basis of clinical and hematologic features. The main types of leukemias are acute lymphocytic leukemia, acute myelogenous leukemia (and subtypes acute myelomonocytic, monocytic, and promyelocytic leukemia), chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia (leukemic reticuloendotheliosis).

ACUTE LEUKEMIAS

The acute leukemias are characterized by a block in maturation of lymphoid or myeloid cells at the primitive blast stage. The immature leukemic cells accumulate in the bone marrow, the peripheral blood, and at times in other tissues. This results in suppression of normal hematopoietic function of the bone marrow, with development of anemia, thrombocytopenia, and granulocytopenia. Patients with large leukemic cell masses and high peripheral blood blast cell counts may develop tissue infiltration of the central nervous system, lungs, liver, spleen, and other organs. The acute leukemias are usually rapidly progressive, death often occurring in a few weeks in untreated patients. Modern combination chemotherapy has improved survival in these diseases, especially in acute lymphocytic leukemia in children; a significant number of patients are now being permanently cured of what was formerly an invariably fatal disease.

Immunologic Features

The cause of leukemia in humans is unknown. Accumulating evidence continues to implicate RNA viruses. Other factors may also be important. Acute leukemia develops in a significant number of individuals exposed to ionizing radiation and some chemicals. Genetic factors are also involved, since leukemia is more frequent in some individuals with chromosomal abnormalities. Improved techniques for the analysis of chromosomes in cultured specimens of bone marrow have yielded results suggesting that all leukemic cells have an abnormal karyotype. Chromosomal abnormalities can also be detected in clinically normal family members.

The acute leukemias are classified by standard morphologic descriptions in the FAB (French, American, British) system. The myeloid leukemias are classified as M1–M7, depending on the type and degree of differentiation. In lymphoblastic leukemia, the terms L1–L3 are used. In the L1 type, most of the cells are small cells with scanty cytoplasm and regular round or cleft nuclei with indistinct nucleoli. In the L2 type, the cells are larger, with more cytoplasm and oval to round nuclei, some with clefts and folds. The chromatin pattern is fine, with prominent nucleoli. In the L3 type (also called Burkitt cell leukemia), the blasts are larger and homogeneous, with finely stippled nuclei and deeply basophilic and vacuolated cytoplasm.

The leukemic cells in acute lymphocytic leukemia (ALL) can be characterized by T and B cell markers (see Chapter 18) and by results of testing with heterologous or monoclonal antisera against T cells, B cells, and non-T, non-B (common) ALL cells. Human B cells are recognized by the presence of readily detectable membrane immunoglobulins and the presence of membrane receptors for C3. Pre-B cells have cytoplasmic but not membrane-bound immunoglobulins and react with antisera against early B cell determinants (eg, B1). Null cells lack T and B cell markers and also fail to react with common-ALL antisera (CALLA).

Acute leukemia cells can also be characterized by the presence of Ia antigen on the cell surface and the presence of the cytoplasmic enzyme marker terminal deoxynucleotidyl transferase (TdT) (see Chapter 18). The latter is found in immature T cells, in most acute

lymphocytic leukemia cells, and in the cells of patients with acute lymphoid transformation of chronic myelogenous leukemia but not in nonlymphoid leukemias or acute myeloid transformation of chronic myelogenous leukemia. Acute lymphocytic leukemia can be classified using a combination of these immunologic techniques, as shown in Table 22–6. Most patients have common-ALL (50–65%) and null-ALL (15–40%), although about 30% of these have pre-B cell characteristics and a lesser percentage have pre-T cell characteristics. About 20% of patients have T-ALL; these patients are important to recognize clinically, because they have a higher incidence of mediastinal tumors and central nervous system involvement and generally have more aggressive disease and a worse prognosis. B-ALL is the least common type of acute lymphocytic leukemia (2–5%) and has a worse prognosis than common-ALL.

Studies with molecular biologic techniques are increasing the ability to characterize early stages of lymphoid cell differentiation in leukemic and other cell populations. Chapter 5 showed that the genes which code for immunoglobulins (including V, J, and H) are strung out linearly on different chromosomes. An extensive rearrangement of these genes occurs early in the development of the cell. This immunoglobulin gene rearrangement is now accepted as the earliest indication that the cell is of B lineage and destined to synthesize immunoglobulin. Molecular probes can detect this immunoglobulin gene rearrangement before synthesis of detectable whole immunoglobulin and thus can identify a leukemic cell as an early pre-B cell. Many cases of ALL previously termed "common" or "null" have now been identified as pre-B cell ALL.

Whereas immunoglobulin genes and cell surface immunoglobulin have been recognized for many years, only in the past few years have the techniques of molecular biology permitted the characterization of the T cell receptor for antigen (see Chapter 11). Several groups have confirmed that the gene which codes for the β chain of the T cell receptor undergoes somatic rearrangement early in T cell development. Molecular probes that detect T_β gene DNA rearrangement are highly specific and act as markers for both lineage (T, B, or other) and clonality.

These probes for immunoglobulin and T_β gene rearrangement are being applied to an increasingly wide array of clinical areas, eg, leukemia, lymphoma, lymph node hyperplasia with unclear evidence for malignancy, lymphocytic infiltrates in AIDS patients, and lymphocytic infiltrates in patients with transplants.

The understanding of the immunologic heterogeneity of acute lymphocytic leukemia has led to a better understanding of the biology of acute lymphocytic leukemia and has contributed to the development of better treatment strategies. For example, the propensity of T cells to migrate to extramedullary sites, such as the meninges and testes, explains the increased frequency of central nervous system and other extramedullary relapses in patients with T-ALL. Also, patients with T-ALL and B-ALL do poorly when treated with standard chemotherapy when compared to patients with common-ALL, and these patients require more intensive chemotherapy. Patients with common-ALL and favorable prognoses, on the other hand, may actually require less intensive therapy than they are now receiving.

Several immunologic tests may indicate the overall prognosis in acute leukemia. A poorer prognosis is suggested by abnormalities in specific immunity, eg, greater amounts of antileukemia antibody and leukemia-associated antigens, and abnormalities in general immune function, eg, reduced lymphocyte activation by PHA or reduced skin test reactivity. Patients with T-ALL have a high T lymphocyte blood count and a poor prognosis. However, patients with acute lymphocytic leukemia and a markedly reduced normal residual T cell count also have a poor prognosis, probably reflecting a reduction in cellular immunity.

Clinical features and therapy are discussed in detail in hematology texts and will not be described here.

CHRONIC LEUKEMIAS

The chronic leukemias are also disorders of cell maturation, resulting in accumulation of abnormal leukemic cells in the bone marrow, peripheral blood, spleen, liver, lymph nodes, and occasionally other organs. Chronic leukemia cells are, however, morphologically and functionally better differentiated than acute leukemia cells, and in general the chronic leukemias are clinically much less aggressive, at times requiring little or no treatment. The chronic leukemias of immunologic interest arise from lymphoid cells; those of myeloid origin will not be discussed in detail.

It is noted that within a mean of 3 years from diagnosis, over half of patients with chronic myelogenous

Table 22–6. Immunologic classification of acute lymphocytic leukemia (ALL).

Type of Acute Lymphocytic Leukemia	Surface Immunoglobulins	Sheep Red Blood Cell Rosettes	Anti-T Antisera	Anti-Common-ALL Antisera	Ia Antigen	TdT
Common-ALL	–	–	–	+	+	+
Null-ALL	–	–	–	–	+/–	+
T-ALL	–	+/–	+	–	–	+
B-ALL	+	–	–	–	+	–

leukemia undergo transformation to acute leukemia, which in most cases is indistinguishable from acute myelogenous leukemia. However, about one-third of patients with typical Philadelphia chromosome-positive chronic myelogenous leukemia appear to undergo transformation to ALL. Evidence for this is based on morphologic, histochemical, and biochemical criteria, particularly the finding of terminal deoxynucleotidyl transferase.

1. CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia is characterized by the progressive accumulation of small lymphocytes of abnormally long life span in blood, bone marrow, liver, spleen, lymph nodes, and other tissues. The leukemia cell in chronic lymphocytic leukemia most often appears as a small but otherwise morphologically normal lymphocyte with a low mitotic rate. Occasionally, larger cells with more abundant cytoplasm are seen resembling those in infectious mononucleosis. Chronic lymphocytic leukemia increases in frequency with age and is often diagnosed by examination of a routine blood film. In many cases it is a relatively benign, slowly progressive disease, causing no symptoms for years. In other patients, however, it may be a serious illness, causing recurrent infections and early death.

The leukemia cell in chronic lymphocytic leukemia is a B lymphocyte in about 95% of cases. In the remainder, the cell is usually a T cell. In most B cell cases there is evidence that the abnormal cells come from a single clone. IgM is the most common monoclonal surface immunoglobulin.

Deficiency of serum immunoglobulins may develop with resultant recurrent infections. A reduction in the serum IgM level often occurs first, followed by reduction of serum IgG and IgA. Abnormalities of both B and T cell function have been found in these patients.

A serum monoclonal immunoglobulin, most frequently IgM, may be found. Generally, however, the concentration of IgM monoclonal protein is not great, and the clinical features of Waldenström's macroglobulinemia do not occur. There may be biclonal gammopathy.

Autoimmune hemolytic anemia develops in some patients with chronic lymphocytic leukemia and frequently is clinically severe. Over half of the patients who become anemic have autoimmune hemolytic anemia with a positive Coombs test. Some patients with chronic lymphocytic leukemia have autoimmune thrombocytopenia as well.

Owing to the concurrent immunodeficiency, patients with chronic lymphocytic leukemia have an increased tendency to develop cancers of other types.

Many asymptomatic patients with chronic lymphocytic leukemia do not require treatment. The onset of symptoms, bulky lymphadenopathy, marked splenomegaly, a rapidly rising lymphocyte count, or a falling red cell and platelet count generally warrants treatment. Chemotherapy usually consists of intermittent or continuous administration of chlorambucil in low doses, although intermittent high doses of chlorambucil-prednisone in combination are also employed. The development of complications requires special treatment, eg, increased prednisone dosage for autoimmune hemolytic anemia or thrombocytopenia. Injections of therapeutic human γ-globulin (ISG) may be necessary in patients with secondary hypogammaglobulinemia and significant recurrent bacterial infections. Rarely, total body irradiation may be used in patients who have developed an acute aggressive form.

2. HAIRY CELL LEUKEMIA (Leukemic Reticuloendotheliosis)

Hairy cell leukemia is being diagnosed with increased frequency. It affects mainly older adult males, who present with fatigue, malaise, infection (often with atypical organisms), abdominal discomfort, pancytopenia, splenomegaly, and, at times, lymphadenopathy. The main pathologic findings are in peripheral blood, bone marrow, spleen, and liver. Pancytopenia is often due to hypersplenism, with monocytopenia a key feature. The number of abnormal cells in the peripheral blood is variable and may be quite low when the white blood cell count is low. The characteristic cell in blood and tissue is shown in Figs 22–3 and 22–4. Routine light microscopy shows the irregular fingerlike projections of cytoplasm that give rise to the term hairy cell leukemia, and these are confirmed by transmission electron microscopy. Scanning electron microscopy further emphasizes the presence of these projections and shows the rufflelike ridges on the surface. The origin of these cells remains controversial. Studies of lymphocyte surface markers, phagocytosis, glass adherence, surface immunoglobulin production, metabolism, in vitro growth, ability to produce colony-stimulating factor, and support of growth of normal bone marrow in vitro have led different groups of investigators to the conclusion that hairy cells are either of B lymphocyte or monocyte-histiocyte origin. Since results have differed among different patients studied, it is possible that the cell of origin in hairy cell leukemia is a primitive stem cell with the capacity to differentiate into both B lymphocyte and monocyte-histiocyte.

The prognosis in hairy cell leukemia is quite variable, but median survival is at least 5 years. Patients with splenomegaly and pancytopenia should be treated by splenectomy. Splenectomy often provides long remissions. Chemotherapy should be reserved for patients who fail to respond to splenectomy or who relapse after a transient response to splenectomy. Recent experiences with interferon therapy have been very encouraging, and it may become the definitive mode of therapy. Death due to infection is a recognized complication of chemotherapy.

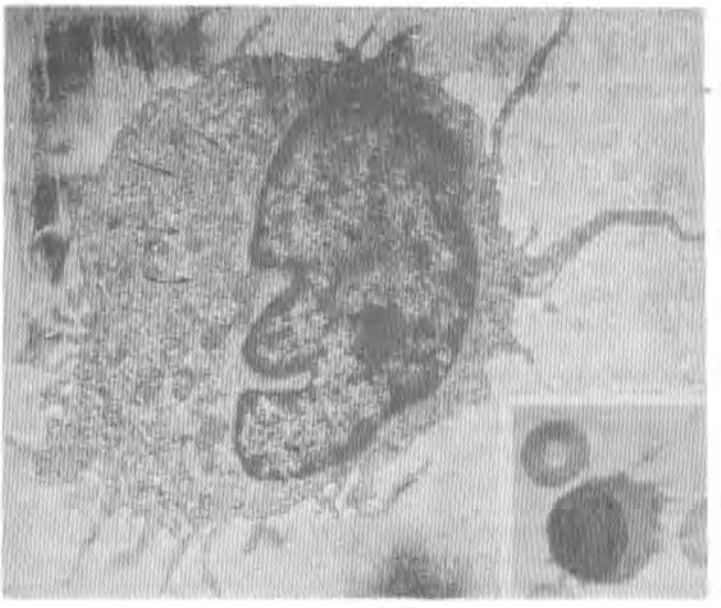

Figure 22–3. Electron micrograph of the neoplastic hairy cell in peripheral blood in leukemic reticuloendotheliosis. (Original magnification × 10,000.) Note the long cytoplasmic projections and multiple ribosome-lamella complexes in the cytoplasm (arrows). The inset is a light photomicrograph of a hairy cell from a peripheral blood smear. (Wright-Giemsa stain; original magnification × 1300.) (Reproduced, with permission, from Katayama I, Li CY, Yam LT: Ultrastructural characteristics of the "hairy cells" of leukemic reticuloendotheliosis. *Am J Pathol* 1972;67:361.)

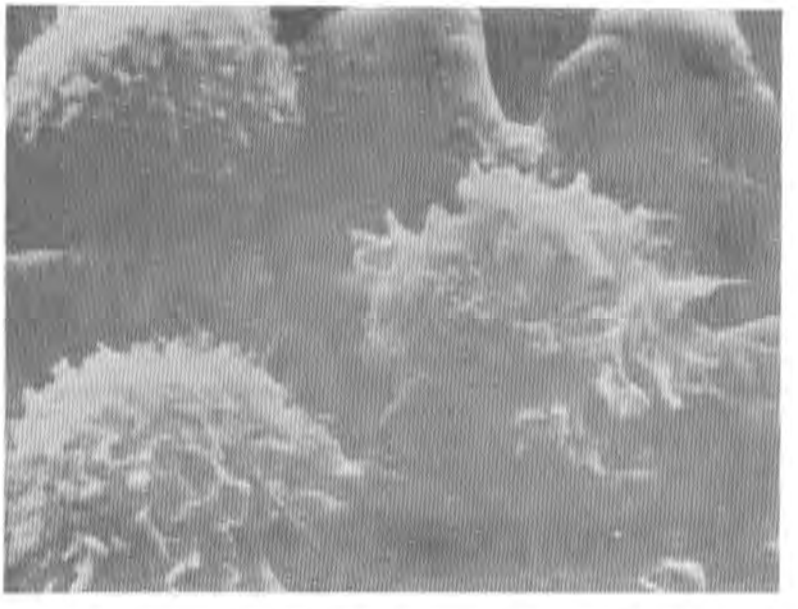

Figure 22–4. Scanning electron micrograph of 2 hairy cells in leukemic reticuloendotheliosis, demonstrating the exaggerated ruffled membranes, ridgelike profiles, and occasional microvilli, resembling monocyte-macrophage cells. A spherical lymphocyte with microvilli is seen in the upper left corner. (Original magnification × 9025.) (Reproduced, with permission, from Polliack A, De Haryen E: An interpretative review: Surface features of normal and leukemic lymphocytes as seen by means of scanning electron microscopy. *Clin Immunol Immunopathol* 1975;3:412.)

LYMPHOMAS

Lymphomas are malignant tumors derived from lymphoid cells, their lymphocytic and histiocytic derivatives, or a combination of these cell types. They represent solid tumors of the immune system, and immunologic abnormalities are common. Lymphomas had been traditionally studied by conventional clinical and histologic methods, but the application of immunologic principles and techniques has greatly improved our knowledge.

Etiology

There are several examples of viruses causing lymphomas in animals, including nonhuman primates. A viral etiology has been proposed for human lymphomas, but causative viruses have not been isolated nor infectivity confirmed. The best-studied example is Epstein-Barr virus (EBV) in Burkitt's lymphoma and X-linked lymphoproliferative syndrome (discussed below with infectious mononucleosis). It may be that viruses exist in lymphomas not as infectious virions but only as part of the viral genome incorporated into the DNA of host cells.

Lymphoma may represent the result of imbalance between homeostatic control mechanisms and immune responses. Specifically, it is suggested that a suppressor lymphocyte population is absent or ineffective and the immune response to a virus or other agent is abnormal, permitting development of malignant lymphoid transformation.

Valuable information relevant to this hypothesis is being obtained with the increased recognition of B cell monoclonal proliferation in patients infected with the AIDS virus (HTLV-III/LAV/ARV). This occurs most frequently as non-Hodgkin lymphoma, but cases of chronic lymphocytic leukemia, benign monoclonal gammopathy, and multiple myeloma have also been seen. The relationship between HTLV-III/LAV/ARV, EBV, and the B cell malignancy has yet to be clarified.

Classification & Immunologic Features

Standardized histologic classification of the lymphomas has led to improved understanding of the natural history and treatment of these diseases. The classification of non-Hodgkin lymphomas remains a controversial area of pathology. The Rappaport classification has been widely used in recent years but has been supplemented by a working formulation (Table 22–7). Immunologic studies have shown the vast majority of nodular and diffuse non-Hodgkin lymphomas to be malignant monoclonal tumors of B lymphocytes, in which degrees of differentiation represent degrees of lymphocyte transformation. It now seems clear that the majority of "histiocytes" in histiocytic lymphomas are transformed B lymphocytes and that true malignancies of the macrophage-histiocytic system are rare (Table 22–8). With continued immunologic, histologic, functional, ultrastructural, and biochemical studies of the lymphomas, it is likely that further revisions in classification will occur. Since most clinical information relevant to prognosis and treatment of the lymphomas has been based on histologic classification, the Rappaport system or the more recent working formulation should be used for clinical purposes at the present time.

A. Hodgkin's Disease: Hodgkin's disease is a malignant lymphoma of mixed cell type. The origin of the characteristic Reed-Sternberg cell and its mononuclear variants remains controversial, thereby excluding immunologic classification at present. Different investigators have presented evidence implicating both B and T lymphocytes and the macrophage-monocyte as the cell of origin in Hodgkin's disease. Most studies have pointed to macrophage-monocyte series as the malignant lineage.

All clinical studies of Hodgkin's disease continue to rely on the histologic classification of Lukes and Butler as modified at the Rye Conference and accurate pathologic staging to determine the extent of disease. About 80% of patients with Hodgkin's disease have histologic findings of nodular sclerosis or mixed cellularity. The nodular sclerosing type of Hodgkin's disease is frequently seen in young women, often associated with a mediastinal mass. Mixed cellularity type is seen in older patients, as is lymphocyte depletion. Lymphocyte predominance type is seen in younger patients, is usually limited in extent, and has an excellent prognosis. Lymphocyte depletion type is at the opposite end of the spectrum, usually presenting with widespread disease and constitutional symptoms and having a poor prognosis. Most investigators feel that the lymphocytic infiltrate present in Hodgkin's disease lesions represents host cellular immune response against the tumor and correlates with a more favorable prognosis. Patients with lymphocyte predominance and nodular sclerosing Hodgkin's disease therefore have a strong host immune response to the tumor, patients with mixed cellularity have an intermediate response, and patients with lymphocyte depletion show a failure of response of the immune system to the tumor.

Defects in cell-mediated immunity occur in Hodgkin's disease, even in early stage I or stage II patients. This can be demonstrated by skin testing and in vitro lymphocyte transformation in response to mitogens, antigens, and allogeneic cells. The patients must be tested before chemotherapy or radiotherapy, which are themselves immunosuppressive.

B. Non-Hodgkin Lymphomas: Until recently, non-Hodgkin lymphomas were classified solely by standard histologic methods. The Rappaport classification (Table 22–7) has usually been used for clinical purposes. Under this system, lymphomas originating in lymph nodes are classified as lymphocytic, histiocytic (previously reticulum cell sarcoma), or mixed and are further subdivided according to degree of differentiation and whether there is a nodular or diffuse histologic pattern. The histologic

Table 22–7. A working formulation of non-Hodgkin lymphomas for clinical use, compared with the Rappaport classification.

New Code	Working Formulation	Rappaport Classification
Low-grade lymphoma		
A	Malignant lymphoma: small lymphocytic cell Consistent with CLL Plasmacytoid	Lymphocytic, well differentiated, diffuse, with and without plasmacytoid features
B	Malignant lymphoma: follicular small cleaved cell Diffuse areas Sclerosis	Lymphocytic, nodular, poorly differentiated
C	Malignant lymphoma: follicular mixed small cleaved and large cell Diffuse areas Sclerosis	Nodular, mixed, lymphocytic and histiocytic
Intermediate-grade lymphoma		
D	Malignant lymphoma: follicular large cell Diffuse areas Sclerosis	Nodular, histiocytic
E	Malignant lymphoma: diffuse small cleaved cell Sclerosis	Diffuse, poorly differentiated, lymphocytic
F	Malignant lymphoma: diffuse mixed small and large cell Sclerosis Epithelioid cell component	Diffuse, mixed, lymphocytic and histiocytic
G	Malignant lymphoma: diffuse large cell, cleaved or noncleaved Sclerosis	Diffuse, histiocytic
High-grade lymphoma		
H	Malignant lymphoma: large cell immunoblastic Plasmacytoid Clear cell Polymorphous Epithelioid cell component	Diffuse, histiocytic
I	Malignant lymphoma: lymphoblastic Convoluted cell Nonconvoluted cell	Lymphoblastic, with and without convolutions
J	Malignant lymphoma: small noncleaved cell Burkitt type Follicular areas	Diffuse; undifferentiated Burkitt and non-Burkitt types

patterns of the majority of patients with non-Hodgkin lymphomas are nodular poorly differentiated lymphocytic or nodular mixed lymphocytic-histiocytic (both with good prognosis), or diffuse poorly differentiated lymphocytic, histiocytic, or mixed (all with poor prognosis).

The working formulation probably will replace the Rappaport system for clinical purposes. Although these histologic classifications have great clinical relevance, they provide little information regarding the origin or biology of these tumors. Immunologic classification (Table 22–8), as proposed by Lukes and Collins, emphasizes the principal immunologic cell type in the lymphoma as well as considering standard histologic features. The degrees of differentiation of cells are viewed as stages in transformation of B lymphocytes. A brief discussion of non-Hodgkin lymphomas is provided, using the working formulation.

1. Small lymphocytic cell, consistent with CLL–(Working formulation code A, low-grade.) Lymph node histologic features identical to those of CLL. Generalized lymphadenopathy and hepatosplenomegaly are common, and bone marrow is nearly always involved. Some patients may have a significant plasmacytoid component and occasionally a circulating IgM monoclonal protein. This is the histologic pattern found in Waldenström's macroglobulinemia.

2. Follicular small cleaved cell–(Working formulation code B, low-grade.) Usually occurs in elderly patients and is derived from the lymph node follicular center cell (B lymphocyte). Abnormal clefted lymphoid cells are found in the peripheral blood and bone marrow in over 50% of patients. Median survival time is 5–7 years despite therapy.

3. Follicular mixed small cleaved cell and large cell–(Working formulation code C, low-grade.) Similar to code B (above) but may have a more aggressive course.

4. Follicular large cell–(Working formulation code D, intermediate-grade.) Presents as stage III or IV disease in 75% of patients. Bone marrow, spleen, and liver involvement occur in approximately 25% of patients. Localized disease is potentially curable; overall median survival time is 3–6 years.

5. Diffuse small cleaved cell–(Working formulation code E, intermediate-grade.) Also a B lymphocyte disorder, probably originating from the follicular center cell. It occurs in the elderly and may transform to more aggressive disease. There is generalized lymphadenopathy, and bone marrow involve-

Table 22–8. Immunologic classification of lymphomas (Lukes and Collins).

Undefined cell type
T cell types
Convoluted lymphocyte
Small lymphocyte
Mycosis fungoides and Sézary's syndrome
Immunoblastic sarcoma (of T cells)
B cell types
Small lymphocyte (chronic lymphocytic leukemia)
Plasmacytoid lymphocyte (Waldenström's macroglobulinemia)
Follicular center cell (FCC) types:
Small cleaved
Large cleaved
Small transformed (Burkitt's lymphoma)
Large transformed
Immunoblastic sarcoma (of B cells)
Histiocytic types

ment is common, with progression to major parenchymal involvement. The majority of patients present as stage III or IV disease.

6. Diffuse mixed small and large cell–(Working formulation code F, intermediate-grade.) Similar to diffuse small cell lymphoma except that it is more likely to present as local disease, but it may follow a more aggressive course. In this category may also be included some T cell lymphomas having a mixed cellular composition.

7. Diffuse large cell–(Working formulation code G, intermediate-[?high-] grade.) Probably a disorder of B follicular center cell origin having heterogeneous morphology with cleaved, noncleaved, or pleomorphic cells. It affects all age groups and commonly presents as stage I or II disease. Initially, it may respond well to multiple-agent therapy and does have the potential for cure, but relapse and an aggressive clinical course are more common. Prognosis has improved considerably in recent years, and the disease may be curable in most patients with stage I and II disease and in up to half of patients with stage III or IV disease.

8. Immunoblastic–(Working formulation code H, high-grade.) May be of B or T lymphocyte type. The morphologic characteristics can be variable, but there is not a high correlation between B or T cell origin and morphology. Some may show marked plasmacytoid features with pyroninophilia, whereas others show clear cell or polymorphous variants. Half of patients present with stage I or II disease, and extranodal presentations of disease are not uncommon. The prognosis is poor. Highly aggressive chemotherapeutic regimens are necessary for therapy.

9. Lymphoblastic–(Working formulation code I, high-grade.) Previously known as Sternberg's sarcoma; typically occurs in children and teenagers, with a male predominance. In most cases, the malignant cell is of T lymphoid origin, although null cell and pre-B cell types have been recognized. The typical morphologic appearance is of a diffuse or pseudonodular pattern and a monomorphous cell population of lymphoblasts with convoluted nuclei, inconspicuous nucleoli, a high mitotic index, and T cell markers. A mediastinal mass is present in 60% of patients. Bone marrow involvement and acute lymphoblastic peripheral blood signs occur early in most patients. The disease normally follows a relentless course with a short survival time, with only 15% of patients living more than 2 years. Results improve when more aggressive chemotherapeutic protocols are used. Central nervous system relapse is common.

10. Small noncleaved cell–(Working formulation code J, high-grade.) Commonly occurs in children in tropical Africa (Burkitt's type) but may be sporadic in other areas. The geographic distribution is related to climate, and the disease is etiologically related to EBV. There is a male predominance. The disease shows a predilection for jaws, gonads, abdominal viscera, and the central nervous system; a rare association with leukemia; and uncommon involvement of peripheral lymph nodes and spleen. The disease has a short survival time without treatment but responds well to chemotherapy. Characteristic histologic findings include a starry-sky pattern, histiocytes with engulfed nuclear debris, and uniform, small, undifferentiated lymphoid cells with round and noncleaved nuclei. The non-Burkitt's type occurs in adults, with gastrointestinal presentation common.

CUTANEOUS T CELL LYMPHOMAS

Mycosis fungoides and **Sézary syndrome** are cutaneous T cell lymphomas. Sézary syndrome is essentially the leukemic form of mycosis fungoides in which the malignant cell is a helper T lymphocyte. In both variants there is relative sparing of the bone marrow. The circulating cells have characteristic morphologic features, with grooved and folded nuclei with a small amount of cytoplasm. Mycosis fungoides and Sézary syndrome are typically indolent diseases of the elderly with cutaneous involvement the clinical hallmark (local lesions in mycosis fungoides and generalized erythroderma in late disease or in Sézary syndrome). Peripheral blood and skin biopsy are the sources of material used for diagnosis. Lymph node involvement is found in 75% and visceral involvement in 60%. Local therapy is usually adequate for the lesions in mycosis fungoides; nitrogen mustard, corticosteroids, psoralens, or electron beam may be used. Visceral disease may require systemic chemotherapy. Systemic chemotherapy or leukapheresis is usually necessary for Sézary syndrome.

ANGIO-IMMUNOBLASTIC LYMPHADENOPATHY WITH DYSPROTEINEMIA

This condition bridges the gap between a reactive lymphoid disorder and true malignancy. It is commonly associated with dysproteinemia and a positive

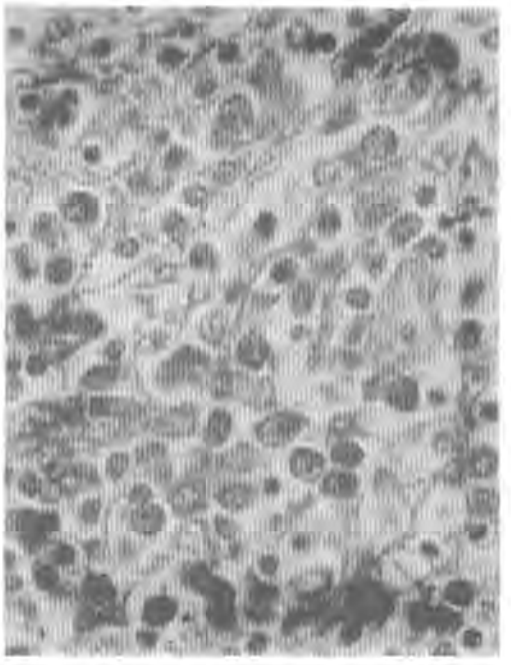

Figure 22-5. Histologic findings in lymph node biopsy of angioimmunoblastic lymphadenopathy. (Giemsa stain, original magnification × 250.) Note the arborizing thick-walled small blood vessels, the amorphous interstitial material, and the mixed cell population, including immunoblasts, plasmacytoid cells, mature plasma cells, and lymphocytes with different intermediate forms. (Reproduced, with permission, from Lukes RJ, Tindle BH: Immunoblastic lymphadenopathy. *N Engl J Med* 1975; *292*:1.

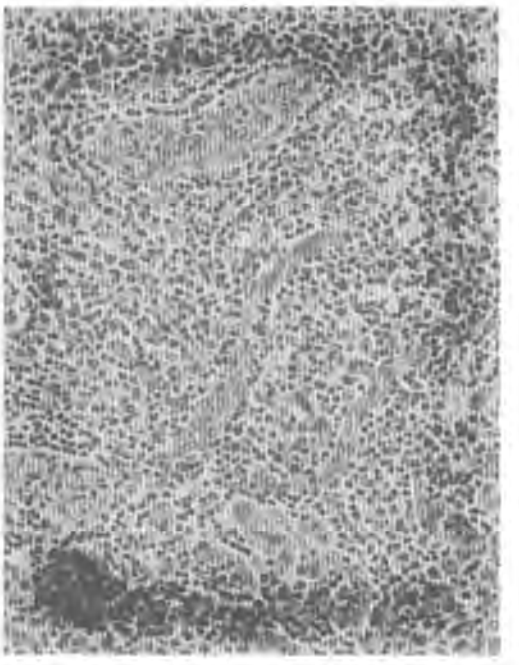

Figure 22-6. Histologic findings in lymph node biopsy of angioimmunoblastic lymphadenopathy which transformed to immunoblastic sarcoma. (Giemsa stain, original magnification × 1000.) Note the cells and numerous immunoblasts. The latter are 15–25 μm in diameter and have large oval nuclei with freely dispersed, pale, basophilic fine chromatin, with one large or 2–3 smaller nucleoli close to the nuclear membrane. (Reproduced, with permission, from Lukes RJ, Tindle BH: Immunoblastic lymphadenopathy. *N Engl J Med* 1975;*292*:1.

Coombs' test. The disease typically occurs in the elderly, and a past history of allergy (especially to drugs) is common. The histologic features include effacement of the lymph node with a pleomorphic infiltrate of immunoblasts and plasma cells. Proliferating and arborizing small blood vessels are typical, and there may be deposits of amorphous interstitial material. Although the disease may evolve into a more classic immunoblastic sarcoma, death from the systemic effects of the disorder and from opportunistic infection is more common. There may be an initial response to corticosteroids, but it is rarely sustained.

TUMORS OF THE MONOCYTE-MACROPHAGE SYSTEM

Now that it is possible to identify definitively the origin of hematologic cancers, tumors of the monocyte-macrophage system have been better categorized. Most of the histiocytic lymphomas of the Rappaport system turned out to be lymphoid in origin (usually follicular center cell lymphomas), and fewer than 5% are true histiocytic cancers.

True Histiocytic Lymphoma (High-Grade)

Clinically, it is usually difficult to delineate true histiocytic lymphomas from diffuse large cell lymphomas without the use of specific cell identification techniques. Fever is common with disease activity, and histiocytic leukemia may be manifested in the terminal stage.

Malignant Histiocytosis (Histiocytic Medullary Reticulosis)

This condition is one of the most fulminant and dramatic of the hematologic malignancies. It has an abrupt onset with systemic symptoms of fever, weight loss, weakness, generalized lymphadenopathy, hepatosplenomegaly, jaundice, and pulmonary and pericardial involvement. Lymph node and bone marrow histologic studies are characteristic but must be differentiated from the recently recognized reactive virus-associated hemophagocytic syndrome. In the lymph node, the sinuses are predominantly involved and exhibit atypical malignant histiocytes, nuclear pleomorphism, multinucleated giant cells, and erythrophagocytosis. The bone marrow may reveal marked hemophagocytosis accounting for the pancytopenia

and hemolytic anemia. Prognosis is poor, and patients rarely survive more than 6 months.

Immunologic Pathogenesis

In most of the non-Hodgkin lymphomas, there are varying degrees of immune paresis and monoclonal protein production.

A. Serum Immunoglobulins: The presence of monoclonal proteins is of course one of the diagnostic requirements in multiple mycloma and Waldenström's macroglobulinemia. However, serum monoclonal proteins are also found in chronic lymphocytic leukemia and in various forms of lymphoma. Monoclonal IgM peaks were found in 5% of lymphomas with a diffuse infiltration of lymph nodes. In some cases the monoclonal protein is a cryoglobulin.

Polyclonal hypergammaglobulinemia is a feature of angioimmunoblastic lymphadenopathy but may occur occasionally in other subjects as a response to infection.

Hypogammaglobulinemia implies a poor prognosis in chronic lymphocytic leukemia or lymphoma, generally indicating advanced disease. It not infrequently leads to death from infection.

B. Cellular Immunity: Anergy is a common finding in Hodgkin's disease, but it can occur with any type of lymphoma at an advanced stage. It is more frequent in later than earlier stages but does not always correlate positively with the stage of disease. Other tests such as lymphocyte activation also commonly indicate defects in cellular immunity. This is expressed clinically as infection with fungi, viruses, or opportunistic organisms such as *Pneumocystis carinii*.

C. Autoimmunity: Lymphoma is recognized as a late complication in patients with autoimmune manifestations, eg, systemic lupus erythematosus or Sjögren's syndrome. Moreover, some patients with lymphoma may subsequently develop autoimmune abnormalities, eg, autoimmune hemolytic anemia in chronic lymphocytic leukemia or angioimmunoblastic lymphadenopathy, immune thrombocytopenic purpura, or other rheumatoid diseases such as dermatomyositis. Immune complex glomerulonephritis has also been reported.

Treatment

The patient with Hodgkin's disease or non-Hodgkin lymphoma must be fully evaluated before treatment can be logically planned. This evaluation, called staging, is used to determine the extent of involvement by lymphoma. Since radiation therapy is often curative when disease is limited to lymph nodes, it is particularly important to evaluate nonnodal tissues such as bone marrow, liver, spleen, etc, for possible involvement. This may require a "staging laparotomy" with resection of multiple lymph nodes from various intra-abdominal and retroperitoneal areas, splenectomy, open liver biopsy, and open bone marrow biopsy. The development of newer methods of noninvasive investigation (eg, gallium scans, magnetic resonance imaging) and the increasing use of fine-needle biopsy will probably further reduce the number of patients undergoing laparotomy. Readers should consult standard texts for details of management. Patients with unfavorable histologic types are often treated with chemotherapy in addition to radiation therapy even when their disease appears to be confined to lymph nodes, since they often have undetected microscopic extranodal disease.

Patients with non-Hodgkin lymphomas more frequently have involvement of extranodal tissues and therefore are less likely to be candidates for radiation.

Advanced Hodgkin's disease responds very well to multiple drug combination chemotherapy. About a third of patients who achieve complete remission appear to be permanently cured. Well-differentiated and nodular lymphocytic lymphomas also respond very well to chemotherapy; diffuse histiocytic and poorly differentiated lymphocytic lymphomas respond less well and have a poorer prognosis.

The typing of lymphomas as T or B cell lymphomas may have therapeutic applications. For example, cytotoxic monoclonal antibodies have been used experimentally to treat T cell lymphomas.

Further advances in understanding lymphomas will come from typing lymphoma cells with molecular biologic methods.

INFECTIOUS MONONUCLEOSIS

Major Immunologic Features

- Acute EBV infection of B lymphocytes.
- Absolute T cell and B cell lymphocytosis.
- Lymphadenopathy and splenomegaly.
- Sheep cell agglutinin > 1:100.
- Antibodies to EBV antigens.
- Antibodies to other viruses and autoantigens.

General Considerations

Infectious mononucleosis is a common acute, usually self-limited infectious disease caused by Epstein-Barr virus (EBV). It may occur at any age, though the highest incidence is in teenagers and young adults. It is usually spread by respiratory droplet infection in epidemic or sporadic epidemiologic distribution.

Immunologic Pathogenesis

Infectious mononucleosis has traditionally been considered to be a lymphoproliferative disease distinguished from lymphoma only by its tendency to spontaneous regression. Several observations testify to the intense lymphoproliferation. The peripheral blood contains atypical lymphoid cells with a high rate of turnover and DNA synthesis. Histologic examination shows marked lymphoproliferation in almost all lymphoid tissues. Lymph nodes show marked hyperplasia, especially in the T-dependent paracortical areas. Distortion of normal architecture by lymphopro-

liferation and the presence of occasional Reed-Sternberg cells rarely leads to an incorrect diagnosis of lymphoma. Lymphocytes taken during the acute phase proliferate in continuous in vitro cultures and will produce lymphomalike lesions if injected into immunosuppressed animals. These lesions can also be produced by fresh noncultured lymphocytes, and human immunoglobulins may be synthesized.

The lymphoproliferation in infectious mononucleosis is secondary to the entry of EBV into B cells. This induces a small and short-lived increase in B cells, which is followed by a marked, prolonged T cell response. This accounts for the variable findings of T and B cell typing of the atypical lymphocytes at different stages of the disease. These responses produce detectable changes in both cellular and humoral immunity. Decreased delayed skin hypersensitivity to antigens is found during the acute phase. Humoral changes include a polyclonal increase in IgG and synthesis of several antibodies (Table 22–9).

Recent studies suggest that the T cell lymphocytosis that occurs in response to primary EBV infection of B cells is a suppressor T cell response, thereby limiting the B cell proliferation and possibly preventing malignant transformation. Changes also occur in T cell subsets in peripheral blood (T4, T8).

A. Antibodies: The heterophil antibody is the IgM antibody in the Paul-Bunnell test. It agglutinates sheep red blood cells and can be absorbed by preincubation with beef red blood cells but not guinea pig kidney. A slide spot test is now used for screening purposes, and positive reactions are confirmed by means of the Paul-Bunnell test. The antibody titer generally rises after day 3 of the illness, reaches a maximum at 2 weeks, and remains high for approximately 6 weeks.

Low-titer IgM and IgG antibodies to i blood group antigen are present in 20–90% of patients. Autoimmune hemolytic anemia may complicate infectious mononucleosis, and anti-i has been tentatively implicated in its pathogenesis.

Serum antibodies to EBV capsid antigen appear de novo within 7 days of clinical disease, increase in titer, fall gradually, and then remain positive at a low level indefinitely. Antibodies to other EBV antigens appear later and are more transient.

Table 22–9. List of antibodies produced in infectious mononucleosis.

Heterophil antibody
Antibodies to i antigen on red blood cells
EBV-associated antibodies:
Early antigen
Membrane antigen
Viral capsid (cytoplasmic) antigen
Nuclear antigen
Virus-neutralizing antibody
Antibodies to Newcastle disease virus
Rheumatoid factor
Antinuclear antibody (ANA)
Syphilis reagins

B. Epstein-Barr Virus (EBV): The association of this herpesvirus with Burkitt's lymphoma was reported in 1964, and intensive study has provided compelling but not fully confirmatory evidence for the oncogenicity of EBV.

There is a high incidence of antibodies to EBV antigens, especially the early antigen, in Burkitt's lymphoma. High titers of antibodies to early antigen are found in all patients. However, up to 30% of patients with American Burkitt's lymphoma do not have EBV antibodies. Furthermore, antibodies to EBV capsid antigen have been detected in some patients with sarcoidosis, systemic lupus erythematosus, and Guillain-Barré syndrome. Efforts to demonstrate enveloped viral particles, nucleocapsids, or early antigens in Burkitt's lymphoma biopsies are generally negative. The continued production of EBV antibodies might be a general indication of B cell overactivity, somehow induced nonspecifically by the lymphoma, or might indicate that viral replication is continuing elsewhere in the body, as happens with Marek's lymphoma in chickens. Fluorescence studies have now demonstrated EBV membrane antigen on tumor cells.

The application of RNA-DNA hybridization techniques to tumor cells, long-term human lymphoblastoid cell lines, and tissues from animals with lymphomas has demonstrated the wide distribution of the EBV genome. The genome may be detected in cells that do not produce infectious virions, and the relevance of this fact to EBV oncogenicity has yet to be explained. Another major paradox is the association of EBV with 3 distinct entities, ie, a lymphoid malignancy (Burkitt's lymphoma), a self-limited lymphoproliferative disease (infectious mononucleosis), and an epithelial malignancy (nasopharyngeal carcinoma). These differences may be due to other etiologic agents in these 3 diseases.

Recent studies have clearly confirmed that EBV infection is significantly associated with many conditions, as follows: infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma, subclinical EBV infection in children, chronic EBV malaise and lethargy syndrome, atypical lymphoid hyperplasia, pseudolymphoma syndromes in adults, B cell lymphomas, X-linked lymphoproliferative syndrome, lymphoproliferative syndromes in immunodepressed subjects, virus-associated hemophagocytic syndrome, bone marrow aplasia, various cytopenias (red cell aplasia, neutropenia, thrombocytopenia), acquired hypogammaglobulinemia, oral hairy leukoplakia, and possibly birth defects. Several factors determine the outcome of EBV infection in the individual patient, including infection with other viruses, eg, AIDS virus (HTLV-III/LAV/ARV) and oral hairy leukoplakia, atypical lymphoid hyperplasia, or B cell lymphoma. Genetic factors are important in determining the outcome of exposure of an individual to EBV, eg, progressive, combined variable X-linked immunodeficiency (Duncan's disease).

Clinical Features

A. Symptoms and Signs: The most frequent presentation is with fever and sore throat, tender lymphadenopathy, anorexia, malaise, headache, and myalgia. There is discrete, moderately tender lymphadenopathy which often is generalized but may be limited to the neck. Splenomegaly occurs in most patients. A macular, maculopapular, or petechial rash occurs in half of cases, but such rashes occur in almost all patients with infectious mononucleosis who have been given ampicillin. Periorbital edema may also occur.

Symptoms may be referable to specifically involved organ systems: myocarditis, manifested by arrhythmias and congestive heart failure; hepatitis, manifested by hepatomegaly and jaundice; central nervous system involvement, manifested by headache, photophobia, neck stiffness, or, rarely, transverse myelitis; and respiratory involvement, manifested by cough, pain, and dyspnea.

B. Laboratory Findings: There is initial granulocytopenia followed by an absolute lymphocytosis. The lymphocytes include many atypical forms which are often larger, with abundant cytoplasm, and show nuclear and cytoplasmic vacuolization. The atypical lymphocytes are not specific for infectious mononucleosis and occasionally are confused with lymphoblasts of acute lymphocytic leukemia.

Liver function tests usually show evidence of mild hepatocellular dysfunction.

The cerebrospinal fluid may show increased pressure and protein and atypical lymphocytes.

The sheep cell agglutination test is discussed above.

Differential Diagnosis

Viral and streptococcal tonsillitis must be excluded as causes of the exudative tonsillitis. Rubella, toxoplasmosis, and infection with cytomegalovirus may resemble some of the manifestations of infectious mononucleosis. An acute mononucleosislike syndrome has been reported in some patients 3–14 days after primary exposure to AIDS virus (HTLV-III/LAV/ARV).

Treatment

There is no specific treatment. Short-term corticosteroids may be useful for acutely ill patients who are very "toxic" and for some complications such as myocarditis, central nervous system involvement, or acute airway obstruction.

Complications & Prognosis

The complications of infectious mononucleosis include secondary bacterial pharyngitis, rupture of the spleen, autoimmune hemolytic anemia, autoimmune thrombocytopenia, myocarditis, hepatitis, and central nervous system involvement with meningoencephalitis or transverse myelitis. Rarely, one may see fatal fulminant infectious mononucleosis or acquired hypogammaglobulinemia.

Fever generally subsides within 10 days and the lymphadenopathy and splenomegaly within 4 weeks. Occasionally, symptoms may last for up to 3 months, and lethargy and malaise may persist for 6–12 months. The mortality rate is negligible.

Recent reports have suggested the possible existence of a chronic syndrome associated with EBV infection. The syndrome shows persistent or recurrent features of fatigue, fever, pharyngitis, lymphadenopathy, headaches, arthralgia, depression, dyslogia, and myalgia. Most patients have antibody to EBV, suggesting active infection for at least 1 year, with increased levels of antibody to viral capsid antigen and early antigen. It is not yet established why such patients are unable to "clear" an initial infection or protect against reinfection with EBV.

LEUKOPENIA

Leukopenia is defined as a reduction in the number of circulating leukocytes below 4000/μL. Granulocytopenia may be caused either by decreased granulocyte production by the bone marrow or by increased granulocyte utilization or destruction. Decreased granulocyte production occurs in aplastic anemia, leukemia, and other diseases marked by bone marrow infiltration; many drugs also cause leukopenia by this mechanism. Increased granulocyte utilization or destruction occurs in hypersplenism, autoimmune neutropenia, and some forms of drug-induced leukopenia.

AUTOIMMUNE NEUTROPENIA

Autoimmune neutropenia may occur as an isolated disorder or secondary to an underlying autoimmune disease. These patients may be asymptomatic or may have recurrent infections. Antigranulocyte antibodies have been detected by a variety of procedures, including the utilization of anti-immunoglobulin antisera with fluorescence or antiglobulin consumption techniques, functional assays, and cytotoxicity assays. The presence of leukoagglutinins does not correlate well with leukopenia. Bone marrow function is relatively normal in autoimmune neutropenia, with myeloid hyperplasia and a shift to the left in maturation often observed, presumably in response to increased peripheral granulocyte destruction. Cases have also been described in which the autoantibody also suppressed bone marrow myeloid cell growth in vitro and in vivo. Autoimmune neutropenia often responds to splenectomy and treatment with corticosteroids or immunosuppressive drugs.

Autoimmune neutropenia may also be seen in systemic lupus erythematosus, Felty's syndrome (rheumatoid arthritis, splenomegaly, and severe neutropenia), and other autoimmune disorders. There is some evidence that immune neutropenia in these dis-

orders may be caused by adsorption of immune complexes onto the neutrophil membrane with premature cell destruction rather than by an antibody directed at specific neutrophil antigens. Some patients with Felty's syndrome also appear to have depressed granulocyte production by the bone marrow, probably also on an immunologic basis.

DRUG-INDUCED IMMUNE NEUTROPENIA

Although most drugs produce neutropenia by bone marrow suppression, some may cause neutropenia by the attachment of drug-antibody immune complexes to the surface of the granulocytes, with premature cell destruction. This "innocent bystander" mechanism is known to occur in drug-induced immune hemolytic anemia and thrombocytopenia. Cephalothin causes granulocytopenia in approximately 0.1% of patients given the drug, probably by this mechanism.

AGRANULOCYTOSIS

Agranulocytosis is characterized by the total absence of granulocytes and granulocyte precursors from the peripheral blood and bone marrow. This most often results from exposure of the patient to certain drugs, eg, aminopyrine, dipyrone, and phenylbutazone. Patients with agranulocytosis usually present with infections—often serious, life-threatening ones. Prior to the antibiotic era, agranulocytosis was almost invariably fatal. Patients now usually recover with intensive antibiotic treatment and granulocyte transfusions when necessary. Unlike drug-induced aplastic anemia, agranulocytosis usually resolves spontaneously within a few days to a few weeks after discontinuing the offending drug.

Although antigranulocyte antibodies or leukocyte drug-dependent antibodies generally have not been demonstrated in agranulocytosis, there is circumstantial evidence that immunologic damage to peripheral blood and bone marrow granulocytic cells is the mechanism of cell destruction, at least in some cases. Such patients often develop agranulocytosis after taking the responsible drug for weeks or months. If they recover from the agranulocytosis after the drug is discontinued and later are rechallenged with a small test dose of the same drug, acute agranulocytosis occurs immediately, associated with the acute onset of fever, chills, and hypocomplementemia.

II. RED BLOOD CELL DISORDERS

The red cell disorders in which immune processes play an important role are the immune hemolytic anemias, paroxysmal nocturnal hemoglobinuria, and aplastic anemia and related disorders.

IMMUNE HEMOLYTIC ANEMIAS

The immune hemolytic disorders are classified in Table 22–10. The classification is based on the behavioral characteristics of antibodies involved and whether there is a demonstrable underlying disease or not. The clinical picture may be one of an acute self-limiting hemolytic disorder but is more often chronic. Since correct identification of the type of antibody is essential to correct diagnosis in patients with suspected immune hemolytic anemia, the immunologic laboratory investigation of such patients will be discussed before the individual diseases.

Immunologic Laboratory Investigations

There are 2 basic groups of immunologic tests necessary to properly investigate patients with suspected immune hemolytic anemias: (1) tests to detect and characterize antibodies involved in the hemolytic process, and (2) tests to aid in diagnosis of possible underlying disease processes. Tests which define underlying disorders include detection of anti-DNA antibodies and ANA in systemic lupus erythematosus, rheuma-

Table 22–10. Classification of immune hemolytic anemias.

Autoimmune hemolytic anemias
- A. Warm antibody types
  1. Idiopathic warm autoimmune hemolytic anemia (AIHA)
  2. Secondary warm autoimmune hemolytic anemias
     a. Systemic lupus erythematosus and other autoimmune disorders
     b. Chronic lymphocytic leukemia, lymphomas, etc
     c. Hepatitis and other viral infections
- B. Cold antibody types
  1. Idiopathic cold agglutinin syndrome
  2. Secondary cold agglutinin syndrome
     a. *Mycoplasma pneumoniae* infection; infectious mononucleosis and other viral infections
     b. Chronic lymphocytic leukemia, lymphomas, etc
  3. Paroxysmal cold hemoglobinuria
     a. Idiopathic
     b. Syphilis, viral infections

Drug-induced immune hemolytic anemias (partial list of drugs)

Aminosalicylic acid (PAS)	Methyldopa
Antihistamines	Penicillin
Carbromal	Phenacetin
Cephalothin	Pyramidon
Chlorinated hydrocarbons	Quinidine
Chlorpromazine	Quinine
Dipyrone	Rifampin
Insulin	Stibophen
Isoniazid	Sulfonamides
Levodopa	Sulfonylureas
Mefenamic acid	Tetracyclines
Melphalan	

Alloantibody-induced immune hemolytic anemias
- A. Hemolytic transfusion reactions
- B. Hemolytic disease of the newborn

toid factors in rheumatoid arthritis, and monoclonal B cells in chronic lymphocytic leukemia.

The serologic tests used to characterize antibodies in serum and on red cells are basic blood-banking procedures, with the addition of monospecific antisera to identify specific proteins on red cells and titration techniques to precisely quantitate antibody activity. Laboratory evaluation of such patients can be considered as a series of questions: (1) Are the red cells of the patient coated with immunoglobulin, complement components, or both? (2) How heavily are the red cells sensitized? (3) What antibodies are eluted from the red cells of the patient? (4) What antibodies are present in the serum?

Routine screening is performed by means of the direct antiglobulin (Coombs) test by tube or slide agglutination (see Chapter 17) using antisera with broad specificity. Subsequent evaluation requires testing the red cells with dilutions of monospecific antisera, especially antisera to IgG and C3. The activity of the autoantibody is examined at different temperatures to see if the temperature of maximal activity identifies it as a "warm" or "cold" antibody.

False-negative and false-positive results can be obtained in direct antiglobulin tests. Approximately 20% of all patients with immune hemolytic anemias will have a negative or only weakly positive direct antiglobulin test unless the antiserum contains adequate titers of antibodies to complement components, especially C3. A positive direct antiglobulin test may be seen in situations other than autoantibodies on red cells and does not necessarily mean autoimmune hemolytic anemia. Causes of such reactions include the following: (1) antibody formation against drugs rather than intrinsic red cell antigens (see below); (2) damage to the red cell membrane due to infection or cephalosporins, leading to nonimmunologic binding of proteins; (3) in vitro complement sensitization of red blood cells by low-titer cold antibodies (present in many normal individuals) in clotted blood samples stored at 4 °C prior to separation; (4) delayed transfusion reactions; and (5) unknown mechanisms. The above reactions are generally weak and can be differentiated by clinical and detailed serologic studies.

Serologic investigations of the patient's serum and red cell eluates should then answer another series of questions: (1) Are antibodies present? (2) Do they act as agglutinins, hemolysins, or incomplete antibodies? (3) What is their thermal range of activity? (4) What is their specificity?

The patient's serum is tested both undiluted and with fresh added complement against untreated and enzyme-treated pools of red cells. Enzyme treatment enhances the sensitivity of the Ii system or abolishes activity in the case of the Pr system. The tests are run at 37 °C and 20 °C and examined at 1 hour for agglutination and lysis. Cold agglutinin titration at 4 °C is also performed. Red cell eluate is similarly tested.

Specialized tests may be performed to detect antibodies to drugs (eg, penicillin) in cases of drug-induced immune hemolytic anemia.

The specificity of the antibodies is tested at different temperatures with a panel of red cells of different Rh genotypes and with cells of different types in the Ii blood group system (see below).

The results of the serologic investigations are then correlated with clinical and other laboratory investigations to establish a definitive diagnosis.

1. WARM AUTOIMMUNE HEMOLYTIC ANEMIA

Major Immunologic Features

- Positive direct antiglobulin (Coombs) test.
- Associated lymphoreticular malignancy or autoimmune disease may be present.
- Splenomegaly common.

General Considerations

Warm antibody autoimmune hemolytic anemia is the most common type of immune hemolytic anemia. It may be either idiopathic or secondary to chronic lymphocytic leukemia, lymphomas, systemic lupus erythematosus (SLE), or other autoimmune disorders or infections (Table 22–10). The idiopathic form may follow overt or subclinical viral infection.

Clinical Features

A. Symptoms and Signs: Patients usually present with symptoms of anemia and hemolysis. There may also be manifestations of an underlying disease, eg, lymphadenopathy, hepatosplenomegaly, or manifestations of autoimmune disease.

B. Laboratory Findings: Normochromic normocytic or slightly macrocytic anemia is usually present; spherocytosis is common, and nucleated red cells may occasionally be found in the peripheral blood. Leukocytosis and thrombocytosis are often present, but occasionally (especially in SLE) leukopenia and thrombocytopenia are seen. There is usually a moderate to marked reticulocytosis. The bone marrow shows marked erythroid hyperplasia with plentiful iron stores. There is an increase in the serum level of indirect (unconjugated) bilirubin. Stool and urinary urobilinogen may be greatly increased. Transfused blood has a shortened survival time.

Immunologic Diagnosis

The results of the serologic tests discussed above are summarized in Table 22–11. The most common pattern is IgG and complement on red cells, with IgG in the eluate. The eluate generally has no activity if the red cells are sensitized only with complement.

Warm hemolysins active against enzyme-treated red cells occur in 24% of sera, but warm serum agglutinins or hemolysins against untreated red cells are rare. The indirect antiglobulin test (see Chapter 17) is positive in approximately 40% of patients' sera tested with untreated red cells but in 80% of serum samples tested with enzyme-treated red cells. This warm anti-

Table 22–11. Summary of serologic findings in patients with autoimmune hemolytic anemia.*

Disease Group	Red Cells: Direct Antiglobulin Test	Red Cells: Eluate	Serum: Immunoglobulin Type	Serum: Serologic Characteristics	Serum: Specificity
Warm antibody type	IgG 30% IgG + complement 50% Complement 20%	IgG IgG No activity	IgG (rarely also IgA or IgM)	Positive indirect antiglobulin test 40% Agglutination of enzyme-treated red cells 80% Hemolysis of enzyme-treated red cells 24% Aggultination of untreated red cells (20 °C) 20% Agglutination or hemolysis of untreated red cells (37 °C) Very rare	Rh system (often with a "nonspecific" component)
Cold agglutinin syndrome	Complement	No activity	IgM (rarely IgA)	High-titer cold agglutinin (usually 1:1000 at 4 °C) up to 32 °C; monoclonal IgM-κ in chronic disease	Anti-I usually (can be anti-i or anti-Pr)
Paroxysmal cold hemoglobinuria (very rare)	Complement	No activity	IgG	Potent hemolysin also agglutinates normal cells. Biphasic (usually sensitizes cells in cold up to 15 °C and hemolyzes them at 37 °C)	Anti-P blood group

*Modified from Petz LD, Garratty G: Laboratory correlations in immune hemolytic anemias. Page 139 in: *Laboratory Diagnosis of Immunologic Disorders*. Vyas GN, Stites DP, Brecher G (editors). Grune & Stratton, 1975.

body is usually IgG but rarely may be IgM, IgA, or both.

The specificity of antibodies in warm antibody autoimmune hemolytic anemia is very complex, but the main specificity is directed against determinants in the Rh complex (see below). Identification is generally performed by blood banks or hematology laboratories with reference panels of red cells of rare types.

Differential Diagnosis

Congenital nonspherocytic hemolytic anemia, hereditary spherocytosis, and hemoglobinopathies can usually be differentiated by the family history, routine hematologic tests, hemoglobin electrophoresis, and a negative direct antiglobulin test.

Treatment

A. General Measures: Treatment of the primary disease is necessary when autoimmune hemolytic anemia is secondary to an underlying disease process. Blood transfusions may be necessary for life-threatening anemia but should be avoided when possible, since the transfused cells are rapidly destroyed. Careful serologic studies are needed to minimize the risks of serious hemolytic transfusion reactions, and successful cross-matching can be difficult or impossible in this situation. Alloantibodies are more common and are difficult to detect.

B. Specific Measures: Hemolysis can be controlled with relatively high doses of corticosteroids in most patients. The steroids are fairly rapidly tapered and then slowly reduced until the clinical state, hemoglobin level, and reticulocyte count indicate the appropriate maintenance dose. Occasionally it is possible to gradually withdraw steroids completely. Regular monitoring is necessary since relapses often occur in patients in remission.

Monitoring generally includes serologic studies, eg, direct and indirect antiglobulin tests, and these may show improvement with reduced amounts of IgG and complement on red cells and lower antibody titers or a negative antibody test. However, there is no consistent correlation between clinical response and serologic tests; prednisone often induces clinical remissions in patients with warm antibody autoimmune hemolytic anemia in spite of persistently positive direct antiglobulin tests.

If prednisone therapy fails or if unacceptable side effects occur, splenectomy is usually performed. Since splenectomy often produces long-term remissions in patients with idiopathic autoimmune hemolytic anemia, splenectomy is the treatment of choice if hemolysis persists after 2–3 months of corticosteroids. ^{51}Cr-labeled red cell survival studies can be used to identify abnormal splenic red cell sequestration prior to splenectomy; however, clinical remissions may occur after splenectomy even when abnormal splenic sequestration cannot be documented. Continued significant hemolysis or late relapse sometimes occurs after splenectomy and requires therapy with steroids with or without other immunosuppressive agents.

Prognosis

The prognosis of idiopathic warm antibody autoimmune hemolytic anemia is fairly good; however, relapses are not infrequent, and death sometimes occurs. The prognosis of secondary warm autoimmune

hemolytic anemia is determined by the underlying disease, eg, SLE or lymphoma.

2. COLD AGGLUTININ SYNDROMES

These diseases may also be primary or may be secondary to infections or the lymphomas (Table 22–10). The infections include mycoplasmal pneumonia and infectious mononucleosis and other viral infections.

The clinical features are often those of the underlying disease. Cold-reactive symptoms such as Raynaud's phenomenon, livedo reticularis, or vascular purpura are seen in some patients. Hemolysis is generally mild but may occasionally be severe, especially in cases secondary to lymphoreticular malignancy. The onset may be acute in cases secondary to infection. The idiopathic form is generally slow in onset and runs a chronic course in older patients.

These diseases usually are characterized by very high serum titers of agglutinating IgM antibodies which react optimally in the cold. These patients have cold agglutinin titers in the thousands or millions, while normal individuals may have low-titer IgM cold agglutinins, and patients with chronic parasitic infections and most patients with *Ancylostoma* infection have titers up to 1:500. The presence of hemolysis is determined by the thermal range of the cold agglutinin. The high-titer, narrow-thermal-range antibodies will cause acral ischemic symptoms. Some, however, may have a low titer but a thermal range reacting up to 37 °C. The specificity of the IgM is generally anti-I in the Ii system, but occasionally it is anti-i or anti-Pr (Table 22–11). In chronic idiopathic cases or cases associated with lymphoreticular malignancy, the cold agglutinin is generally a monoclonal IgM-κ paraprotein. The direct antiglobulin test is always positive using antiserum to C3.

Treatment consists of keeping the patient warm and waiting for spontaneous resolution in acute cases. Chronic cases sometimes respond to chlorambucil in low doses. Corticosteroids and splenectomy are probably not helpful, unless an underlying lymphoma is present.

The prognosis is generally good except for patients with severe underlying disease such as malignant lymphoma.

3. DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

Many cases of immune hemolytic anemia have been reported in association with drug administration; the most common examples are included in Table 22–10. There are 3 stages in the investigation of a patient with suspected drug-induced hemolytic anemia: a history of intake of the drug, confirmation of hemolysis, and serologic tests. Detailed serologic tests are necessary to confirm the diagnosis, since different drugs produce hemolysis by different mechanisms. The immunopathologic mechanisms and clinical and laboratory features are summarized in Table 22–12.

Table 22–12. Summary of immunopathologic mechanisms and clinical and laboratory features in drug-induced immune hemolytic disorders.*

Mechanism	Drugs	Clinical Findings	Serologic Evaluation	
			Direct Antiglobulin Test	Antibody Characterization
Immune complex formation (drug + antidrug antibody)	Quinine, quinidine, phenacetin	History of small doses of drugs. Acute intravascular hemolysis and renal failure. Thrombocytopenia occasionally found.	Complement (IgG occasionally also present).	Drug + patient's serum + enzyme-treated red cells → Hemolysis, agglutination, or sensitization. Antibody often complement-fixing IgM. Eluate generally nonreactive.
Drug adsorption to red cell membrane (combination with high-titer serum antibodies to drug)	Penicillins, cephalosporins	History of large doses of drugs. Other allergic features may be absent. Usually subacute extravascular hemolysis.	IgG (strongly positive if hemolysis occurs). Rarely, weak complement sensitization also present.	Drug-coated red cells + serum → Agglutination or sensitization (rarely hemolysis). High-titer antibody. Eluate reacts only with antibiotic-coated red cells.
Membrane modification (nonimmunologic adsorption of proteins to red cells)	Cephalosporins	Hemolytic anemia rare.	Positive with reagents with antibodies to a variety of serum proteins.	Drug-coated red cells + serum → Sensitization to antiglobulin antisera in low titer.
Unknown	Methyldopa	Gradual onset of hemolytic anemia. Common.	IgG (strongly positive if hemolysis occurs).	Antibody sensitizes normal red cells without drug. Antibody in serum and eluate identical to warm antibody. No in vitro tests demonstrate relationship to drug.

*Adapted from Garratty G, Petz LD: Drug-induced immune hemolytic anemia. *Am J Med* 1975;58:398.

The mechanisms are classified as immune complex formation, hapten adsorption, nonspecific adsorption, and other, unknown mechanisms.

(1) Immune complex formation: Circulating preformed immune complexes between the drug and antibody to the drug sensitize the red cell ("innocent bystander" phenomenon). Quinine in low doses is a typical example. There is great variability in clinical features and serologic findings.

(2) Drug (hapten) adsorption: The drug acts as a hapten in that it is bound to the red cell membrane and stimulates the production of a high titer of antidrug antibodies.

(3) Nonspecific adsorption: The drug affects the red cells so that various nonimmunologic proteins are adsorbed onto red cells and give a positive Coombs test. This does not result generally in marked hemolysis.

(4) Unknown mechanisms: This type is exemplified by the positive Coombs test that develops within 3 months in 20% of patients treated with methyldopa. The IgG that coats red cells in these patients does not have antibody activity against the drug, and the drug is not required in in vitro tests.

The hemolysis may be acute and severe, but only rarely is blood transfusion required. The main treatment is to stop the offending drug and monitor the patient to be sure the hemolysis disappears. The prognosis is therefore excellent.

4. PAROXYSMAL COLD HEMOGLOBINURIA

This rare disease may be transient or chronic. It may occur as a primary idiopathic disease or secondary to syphilis or viral infection. It is characterized clinically by signs of hemolysis and hemoglobinuria following local or general exposure to cold. Symptoms may include combinations of fatigue, pallor, aching and pain in the back, legs, or abdomen, chills and fever, and the passing of dark-brown urine. The symptoms may appear from within a few minutes to a few hours after exposure to cold.

The disease is characterized by the presence of the classic biphasic Donath-Landsteiner antibody. This IgG antibody sensitizes red cells in the cold (usually below 15 °C), so that complement components are detected on the red cells by the direct antiglobulin test after rewarming. Heavily sensitized cells are hemolyzed when warmed to 37 °C.

Acute attacks are treated symptomatically, and postinfectious cases generally resolve spontaneously.

5. HEMOLYTIC DISEASE OF THE NEWBORN

Immunologic Pathogenesis

During pregnancy, very small amounts of fetal blood are leaked into the maternal circulation, especially during the last trimester. However, this is usually not enough to trigger antibody formation in the mother. During delivery, when the placenta is detached, bleeding of cord blood into the mother's circulation can elicit an immune response to fetal red cell alloantigens.

Hemolytic disease of the newborn results from the mother's antibodies crossing the placenta and destroying fetal red cells. This leads to hemolytic anemia and hydrops in the newborn infant. Hyperbilirubinemia occurs as a postnatal complication.

The first child is seldom affected by the hemolytic disease, but the chances for alloimmunization increase with each incompatible pregnancy. The primary stimulus for immunization can also be a previous incompatible blood transfusion or abortion.

Formation of Rh antibodies is the most common form of alloimmunization to give rise to clinically important disease. Antibodies to blood groups A and B (see Chapter 19) may also cause hemolysis of fetal cells if the maternal antibodies are IgG and thus capable of crossing the placenta. In these cases, the mother belongs usually to group O and the baby to group A. In fact, ABO immunization during pregnancy occurs more often than Rh immunization, but it seldom results in serious problems. If the fetus secretes soluble A or B substances, the maternal antibodies become neutralized before they cause damage to red cells. A or B substances are present not only on red cells but also on other tissues, including the placental endothelium. Therefore, many of the antibodies are consumed by these cells.

Clinical Features

The most frequent signs in the newborn are anemia and rapidly developing jaundice, which is usually present within the first 24 hours (in contrast to the physiologic icterus that occurs later). The infant's response to the anemia is marked reticulocytosis and erythroblastosis. As bilirubin accumulates in the plasma, it may cross the blood-brain barrier and cause damage to the nervous system (kernicterus). Severe alloimmunization causes fetal hydrops, and the fetus may die in utero. In these cases, if the father is homozygous for the relevant blood group, the prognosis is very poor for future babies.

Immunologic Diagnosis

Since the cause of the disease is antibody on red cell membrane, the direct Coombs test is usually positive. In ABO incompatibility, it is often negative. The reason for this is somewhat unclear, but the relatively small amount of IgG antibody and the adsorption by other tissues may result in so few antibody molecules on the red cell surface that the conventional Coombs method is not able to detect them. Thus, a negative direct Coombs test does not rule out an immunologic cause for neonatal icterus. If antibodies are not found in the mother's serum, however, immune hemolysis is unlikely.

Alloimmunization should be detected during preg-

nancy. In many countries, all Rh-negative women are screened for the presence of blood group antibodies during pregnancy. As the number of D immunizations decreases, the relative proportion of immunizations to other blood groups has increased. Consequently, antibody screening should not be restricted to Rh-negative women. No reliable screening test is available for ABO disease, although several assays for detection of clinically important IgG anti-A or anti-B have been used.

When unexpected antibodies are found in the mother's serum, the father's blood groups should be determined. If the father is negative for the relevant blood group, there is no risk; if he is heterozygous, the baby has only a 50% chance of being affected. Increasing antibody titer or a history of previously ill children increases suspicion that the fetus can be affected, and amniocentesis is done to determine the concentration of bile pigments and possibly antibodies in the amniotic fluid. With these procedures and with ultrasound examination, the presence and seriousness of the hemolytic disease can be assessed. Detectable amounts of antibodies sometimes develop in the serum so late in the pregnancy that they remain unnoticed until the time of delivery. Alloimmunization should always be suspected if the bilirubin level starts rising rapidly in an anemic newborn infant.

Treatment & Prevention

Treatment can be started during the last trimester of pregnancy if the results of amniocentesis and antibody determinations indicate that the fetus has serious disease. Compatible blood is injected into the abdominal cavity of the fetus and is rapidly absorbed into the circulation. These intrauterine transfusions may help the fetus to survive until mature enough to live outside the uterus. The last weeks of pregnancy are the most critical time for the fetus. Careful monitoring of clinical data by the obstetrician and neonatologist may prompt a decision to deliver the affected baby prior to term.

Immediately after delivery, the infant's blood group is determined and the cord cells are tested by the direct Coombs technique. If the baby is affected, exchange transfusions are usually needed, although in mild cases phototherapy with ultraviolet light or close supervision of bilirubin levels may be sufficient.

Women who have antibodies to the fetal red cells should deliver in hospitals that have facilities and experience in exchange transfusion. Despite modern advances in the treatment of hemolytic disease of the newborn, the mortality rate in severe intrauterine cases remains high.

Over 90% of Rh-negative women having Rh-positive offspring do not form anti-D antibodies. The immunization of the rest can be prevented by giving the mother concentrated anti-D (Rh_0) immunoglobulin within 72 hours of delivery if she does not have any preexisting anti-D antibodies. Since it is not possible to predict who will make antibodies, all Rh-negative women with an Rh-positive baby must be given prophylaxis. Since Rh antigens are detectable in an embryo a few weeks postconception, anti-D immunoglobulin should also be given to Rh-negative women who have aborted.

The mechanism of inhibition of antibody synthesis is unclear, but rapid destruction and clearance of Rh-positive cells from the circulation seem to play a role. In experimental conditions, Rh-positive cells coated with blood group antibodies other than anti-D are quickly destroyed and anti-D antibodies are not formed. This idea is supported by the clinical observation that mothers with anti-A or anti-B antibodies reacting with fetal cells produce Rh antibodies less often than in ABO-compatible pregnancies.

Systematically applied anti-D prophylaxis has reduced the number of immunized women from about 7–8% to a little over 1% if measured by the number of Rh-negative women with antibodies after 2 consecutive Rh-positive babies. Several reasons have been suggested for the few failures: immunization early during the pregnancy (not starting at the time of delivery); abnormally large volume of fetal blood leaking into the maternal circulation with insufficient anti-D immunoglobulin; and unusual sensitivity of the maternal immune system to the antigen D.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

This rare disease is now known to cover a wide spectrum of clinical presentations. It can occur in adults as a chronic hemolytic anemia with acute exacerbations. It may follow other hematologic disorders such as idiopathic or drug-induced bone marrow aplasia and may terminate in acute myelogenous leukemia. The intravascular hemolysis causes intermittent hemoglobinemia and hemoglobinuria. This activity fluctuates throughout the day; the classic timing with nocturnal hemoglobinuria is seen in only 25% of cases. Venous thrombosis is a recognized complication.

The diagnosis is suggested by the findings of intermittent or chronic intravascular hemolysis, iron deficiency, hemosiderinuria, a low leukocyte alkaline phosphatase value, and frequently pancytopenia. The diagnosis of paroxysmal nocturnal hemoglobinuria is confirmed by any of the following tests: the acid hemolysis (Ham) test, the sugar water test, and the inulin test. These tests are presumably expressions of the 2 presently known abnormalities in paroxysmal nocturnal hemoglobinuria, ie, the exquisite sensitivity of paroxysmal nocturnal hemoglobinuria red cells to complement lysis and the abnormally low acetylcholinesterase activity in the red cell membrane. Patients' cells are lysed by approximately 4% of the amount of complement required to lyse normal red cells. These tests demonstrate the sensitivity to complement lysis of paroxysmal nocturnal hemoglobinuria red cells but do not elucidate the fundamental underlying cause of paroxysmal nocturnal hemoglobinuria. The alternative complement pathway may well be involved in this

disease, as suggested by the inulin test, since inulin activates this pathway. However, serum complement studies are normal, no antibody has been identified either in the serum or on red cells, and no abnormalities have been defined in membrane lipids and phospholipids. Electron microscopy shows a pitted surface on red cells, but this has not been correlated with the functional complement abnormalities.

Treatment is mainly symptomatic. Transfusions are often required, and reactions are not infrequent. Androgens may be useful if there is underlying bone marrow hypoplasia. Corticosteroids and splenectomy are probably not useful. Rarely, bone marrow transplantation may be possible.

APLASTIC ANEMIA & RELATED DISORDERS

Recently there has been growing interest in the possibility that aplastic anemia and certain other disorders characterized by lack of normal hematopoiesis by the bone marrow may be immunologic in origin. Reports have confirmed immunologic mechanisms in pure red cell aplasia and some patients with aplastic anemia.

Pure Red Cell Aplasia

This rare form of anemia is characterized by a marked reduction or absence of bone marrow erythroblasts and blood reticulocytes, with normal granulopoiesis and thrombopoiesis. It occurs as an acquired disorder in adults, either in an idiopathic form or associated with thymoma, lymphoma, other tumors, or certain drugs. Patients usually present with progressive anemia, generally requiring transfusion support. Bone marrow examination confirms the diagnosis. Thymoma is present in a small number of patients. Other immunologic abnormalities, such as hypogammaglobulinemia, monoclonal gammopathy, autoimmune hemolytic anemia, myasthenia gravis, and features of SLE are being seen in increasing numbers of patients with pure red cell aplasia.

Many patients with pure red cell aplasia, with or without thymoma, have been found to have serum antibodies that react with bone marrow erythroblasts. These IgG antibodies have been demonstrated by immunofluorescence microscopy, with staining of nuclei of bone marrow erythroblasts. These antibodies fix complement and are specifically cytotoxic for erythroblasts. It has also been demonstrated that plasma from patients with pure red cell aplasia suppresses erythropoiesis by normal bone marrow when cultured in vitro, while bone marrow from patients with pure red cell aplasia shows normal erythropoiesis when cultured in vitro in normal plasma. This plasma factor suppressing erythropoiesis in pure red cell aplasia is an IgG antibody.

Patients with pure red cell aplasia usually require total red blood cell transfusion support. Patients with thymomas should have these tumors removed; this will produce a remission in about 30% of these patients. Patients with idiopathic pure red cell aplasia and those who do not respond to thymectomy should be treated with immunosuppressive drugs. Corticosteroids are usually used first, but few patients respond, and most are subsequently treated with cyclophosphamide plus prednisone. This combination produces remissions in 30–50% of patients, but relapses may occur when drugs are discontinued. Splenectomy has also been advocated for refractory patients with pure red cell aplasia, as has plasma exchange.

Diamond-Blackfan Syndrome

This disorder, also known as congenital hypoplastic anemia, represents the congenital form of pure red cell aplasia seen in infants. Anemia is usually noted in the first year of life but may occur later. These patients must be distinguished from patients with transient erythroblastopenia of infancy and childhood, which is a less serious self-limited disorder.

Aplastic Anemia

Aplastic anemia is defined as pancytopenia due to bone marrow aplasia. Patients with severe aplastic anemia have no hematopoietic precursor cells present in their bone marrow and must be supported with red cell and platelet transfusions and antibiotics. Problems exist with continuing transfusion support; even with optimal supportive care, severe aplastic anemia rarely undergoes spontaneous remission, and there is a 75–90% mortality rate. Treatment with high doses of androgens may benefit some patients, but few patients with severe aplasia respond. Bone marrow transplantation produces long-term remissions in over 50% of patients with severe aplastic anemia, and early bone marrow transplantation is currently considered the treatment of choice for younger patients with a histocompatible matched sibling.

In the past, aplastic anemia was usually associated with exposure to toxic drugs or chemicals (benzene, chloramphenicol, arsenicals, gold, anticonvulsants, etc). Recent series, however, indicate that most patients have no such exposure and no other associated illness, and these patients are classified as having idiopathic aplastic anemia. Although it is possible that these patients have been exposed to unknown or inapparent environmental toxins, recent studies indicate that at least some of these cases are due to immunologic causes. Lymphocytes from the bone marrow of about one-third of patients with aplastic anemia have been shown to suppress the growth of granulocyte colonies from normal bone marrow in vitro. When these abnormal suppressor lymphocytes are separated from the marrow granulocytic stem cells or killed with a specific cytotoxic antilymphocyte serum, increased granulocyte colony formation occurs. Other investigators found that peripheral blood lymphocytes from patients with aplastic anemia may suppress erythropoiesis of normal bone marrow when cultured in vitro.

Good therapeutic responses to antithymocyte globulin (ATG) have been noted, supporting an immune

pathogenesis for aplastic anemia. Caution is required in the use of ATG in this setting, however, as multiple myeloma has been reported in a patient with aplastic anemia responding to 2 courses of ATG.

Aplastic anemia, therefore, may result from at least 3 different defects involving the stem cells, the hematopoietic environment, or suppressor cells. A review of 14 patients with aplastic anemia studied by in vitro bone marrow cultures found evidence that 8 patients had defects in their stem cells. One patient had a defective hematopoietic environment, and 5 patients had increased suppressor cell activity. Characterization of the nature of the defects would permit more rational management of patients with aplastic anemia, since those patients with evidence of increased suppressor cell activity would be considered for treatment with immunosuppressive drugs or ATG and those with obvious stem cell defects would be considered for early bone marrow transplantation.

III. PLATELET DISORDERS

Thrombocytopenia may be due to decreased platelet production, increased platelet destruction, or abnormal platelet pooling. Immunologic thrombocytopenias, the subject of this section, are caused by increased platelet destruction, usually following platelet sensitization with antibody. Thrombocytopenias due to decreased platelet production (aplastic anemia, leukemias, etc) have already been discussed with regard to immunologic features. Thrombocytopenia due to abnormal platelet pooling in an enlarged spleen (hypersplenism) is generally not associated with immunologic abnormalities.

Immunologic Mechanisms of Platelet Destruction

Several immunologic mechanisms of platelet damage leading to thrombocytopenia have been described. Platelet autoantibodies sensitize circulating platelets in idiopathic thrombocytopenic purpura and related disorders, leading to premature destruction of these cells in the spleen and other parts of the monocyte-macrophage system (see following section). Platelet alloantibodies may develop after multiple transfusions with blood products, or maternal sensitization can occur during pregnancies. Such platelet alloantibodies are becoming a major problem in long-term platelet support for patients with bone marrow failure. Alloantibodies may cause shortened platelet survival after transfusion or produce immediate platelet lysis with severe fever and chill reactions. Shortened platelet survival appears to be mediated by non-complement-dependent IgG or IgM antibodies similar to autoantibodies seen in idiopathic thrombocytopenic purpura. Platelet lysis, on the other hand, appears to be mediated by complement-dependent cytotoxic antibodies. These alloantibodies are directed primarily at HLA antigens, but non-HLA platelet antigens may also be involved. Alloantibody-dependent lymphocyte-mediated cytotoxicity has also been described in some patients. Neonatal thrombocytopenia due to passive transfer of maternal alloantibody or autoantibody is fortunately rare but may be life-threatening when it occurs.

Other immunologic mechanisms of platelet destruction include development of antibodies to drugs or other antigenic substances (haptens) adsorbed to the platelet membrane and adsorption of preformed antigen-antibody complexes onto the platelet membrane, with rapid removal of these sensitized cells from the circulation ("innocent bystander" phenomenon). The reactions are often complement-dependent. These mechanisms are seen in drug-induced immune thrombocytopenia, in some infections, and in autoimmune disorders such as SLE. It has been suggested that cell-mediated immunity, ie, lymphocyte activation, may alone be able to cause platelet damage and thrombocytopenia. Lymphocyte activation has been observed in response to autologous platelets in some patients with idiopathic thrombocytopenic purpura. Whether this represents a true cellular immune response or whether the lymphocytes are reacting to immune complexes or otherwise altered platelets remains uncertain. Finally, it is known that bacterial endotoxin can cause thrombocytopenia directly, usually involving activation of the complement system. Antibodies are not required for this reaction.

Table 22–13 shows a classification of immunologic thrombocytopenias that are discussed in more detail in the following section.

IDIOPATHIC THROMBOCYTOPENIC PURPURA

Major Immunologic Features

- Antiplatelet antibodies demonstrable on platelets and in serum.
- Shortened platelet survival.
- Therapeutic response to prednisone and splenectomy.

General Considerations

Idiopathic thrombocytopenic purpura is an autoimmune disorder characterized by increased platelet destruction by antiplatelet autoantibody. IgG autoantibodies sensitize the circulating platelets, leading to accelerated removal of these cells by the macrophages of the spleen and at times the liver and other components of the monocyte-macrophage system. Although there is a compensatory increase in platelet production by the bone marrow (total platelet turnover may be 10–20 times the normal rate), thrombocytopenia occurs, and, depending on the severity, gives rise to the 2 typical clinical features of the disease: purpura and bleeding.

Idiopathic thrombocytopenic purpura most often

Table 22–13. Classification of immune thrombocytopenias.

Idiopathic (autoimmune) thrombocytopenic purpura (ITP)	
Secondary autoimmune thrombocytopenias	
Systemic lupus erythematosus and other autoimmune disorders	
Chronic lymphocytic leukemia, lymphomas, some nonlymphoid malignancies	
Acquired immunodeficiency syndrome (AIDS)	
Infectious mononucleosis and some other infections	
Drug-induced immune thrombocytopenias (partial list of drugs)	
Acetazolamide	Imipramine
Allymid	Meprobamate
Aminosalicylic acid (PAS)	Methyldopa
Antazoline	Novobiocin
Apronalide	Phenolphthalein
Aspirin	Phenytoin
Carbamazepine	Quinidine
Cephalothin	Quinine
Chlorothiazide	Rifampin
Digitoxin	Spironolactone
Factor VIII concentrate	Stibophen
Heparin	Sulfamethazine
Hydrochlorothiazide	Thioguanine
Posttransfusion purpura	
Thrombotic thrombocytopenic purpura (TTP)	
Neonatal immune thrombocytopenias	
Due to autoantibodies (ITP)	
Due to alloantibodies (maternal sensitization)	
Due to alloantibodies (destruction of transfused platelets)	
Sensitization from previous transfusions	
Maternal sensitization during pregnancies	

occurs in otherwise healthy children and young adults. Childhood idiopathic thrombocytopenic purpura often occurs within a few weeks following a viral infection, suggesting possible cross-immunization between viral and platelet antigens, or adsorption of immune complexes, or a hapten mechanism. Adult idiopathic thrombocytopenic purpura is less often associated with a preceding infection. An identical form of autoimmune thrombocytopenia can also be associated with SLE, chronic lymphocytic leukemia, lymphomas, nonlymphoid malignancies, infectious mononucleosis, and other viral and bacterial infections. Certain drugs can also cause immune thrombocytopenia, and these can produce a clinical picture that is indistinguishable from idiopathic thrombocytopenic purpura.

Although adult and childhood idiopathic thrombocytopenic purpura appear to have similar basic pathophysiologic features, there are significant differences in their course and therefore their treatment. Most children have spontaneous remissions within a few weeks to a few months, and splenectomy is rarely necessary. Adult patients, on the other hand, rarely have spontaneous remissions and usually require splenectomy within the first few months after diagnosis. Although some authors have attempted to separate acute and chronic idiopathic thrombocytopenic purpura into 2 distinct entities, current clinical, immunologic, and kinetic findings provide little justification for this distinction.

Idiopathic thrombocytopenic purpura has recently been reported in a series of previously healthy homosexual men. High levels of circulating immune complexes were found, and some had reduced helper T/suppressor T cell ratios. The relationship of this disorder to acquired immunodeficiency syndrome (AIDS) is not yet defined.

Immunologic Diagnosis

Harrington and coworkers first showed in 1951 that the plasma from most patients with idiopathic thrombocytopenic purpura contained a factor that caused thrombocytopenia when transfused into normal human recipients. Although the plasma factor was suspected of being an antibody, immunologic tests to detect serum antibodies (agglutination, complement fixation, etc) did not demonstrate the factor, since these tests are too insensitive to detect platelet autoantibody in idiopathic thrombocytopenic purpura and similar disorders. More sophisticated techniques have been developed for detection of antiplatelet antibodies in the past decade (Table 22–14). The so-called immuno-injury techniques (platelet factor 3 release, ^{14}C-serotonin release) detect antiplatelet antibodies in the serum of 60–70% of adult patients with idiopathic thrombocytopenic purpura. Recent methods for detecting platelet-autoantibody complexes by lymphocyte activation or ingestion by granulocytes, or competitive binding assays or antiglobulin tests for the measurement of antiplatelet antibodies on the platelet surface have shown positive results in almost all patients with idiopathic thrombocytopenic purpura. Unfortunately, all of these latter methods are generally too complex for routine clinical laboratory use, so in most cases the diagnosis of immune thrombocytopenia must be made without the benefit of a specific immunologic diagnosis.

Platelet Kinetics

^{51}Cr-platelet kinetic studies show that all patients with idiopathic thrombocytopenic purpura and other

Table 22–14. Tests for platelet autoantibodies in idiopathic thrombocytopenic purpura (ITP).

Methods	Percent Positive
Standard immunologic tests (agglutination, complement fixation, etc)	0
Transfusion of plasma from patients with ITP into normal donors	63–75
Platelet factor 3 release	65–70
^{14}C-serotonin release	60
Lymphocyte activation by autologous platelets	70
Lymphocyte activation by platelet-antibody immune complexes	90+
Phagocytosis of platelet-antibody immune complexes by granulocytes	90+
Measurement of platelet-associated IgG by competitive binding assays	90+
Radiolabeled Coombs antiglobulin test	90+
Fluorescein-labeled Coombs antiglobulin	90+
Enzyme-linked immunosorbent assay (ELISA)	90+

types of autoimmune thrombocytopenia have markedly shortened platelet survival times ($t_{1/2}$ 0.1–30 hours; normal $t_{1/2}$ 100–120 hours) and have normal or only slightly subnormal platelet recoveries at t_0 (40–80%; normal 60–80%). About 75% of patients have splenic platelet sequestration, and 25% have both splenic and hepatic sequestration. Patients with thrombocytopenia due to an enlarged splenic platelet pool can be easily distinguished from patients with autoimmune thrombocytopenia by these kinetic methods. Although it was felt initially that determination of the sites of platelet sequestration might be useful in predicting the response to splenectomy, a recent report shows no significant difference in response rates based on presplenectomy platelet sequestration patterns. Both groups in this report had an 85–90% complete remission rate at 2 years' follow-up postsplenectomy.

Clinical Features

A. Symptoms and Signs: The onset may be acute, with sudden development of petechiae, ecchymoses, epistaxis, and gingival, gastrointestinal, or genitourinary tract bleeding. Alternatively, the disease may be gradual in onset and chronic in course. Often, however, chronic idiopathic thrombocytopenic purpura is slowly progressive or suddenly becomes acute.

B. Laboratory Findings: The platelet count is usually less than 20,000–30,000/μL in acute cases, and 30,000–100,000/μL in chronic cases. There may be moderate anemia due to blood loss and iron deficiency. The white blood count is normal or slightly increased but may be low in SLE. Platelets are often larger than normal on peripheral blood smear, and no immature white cells are present. The bone marrow shows normal or increased numbers of megakaryocytes and is otherwise normal. The megakaryocytes may be normal or immature in appearance but at times are larger than normal with increased numbers of nuclei.

Differential Diagnosis

All causes of thrombocytopenia must be considered when evaluating a patient with suspected idiopathic thrombocytopenic purpura (Table 22–15). Patients with idiopathic thrombocytopenic purpura characteristically feel and look well, and all physical and laboratory findings are normal except for thrombocytopenia and the associated purpura and possible bleeding. Patients with "consumptive" thrombocytopenias, on the other hand, tend to be acutely ill, often with fever and evidence of multisystem disease, especially renal disease. These patients generally have microangiopathic hemolytic anemia, the fragmented red cells being a critical diagnostic finding on the peripheral blood smear. Abnormalities of clotting function are also often present. Patients with acute leukemia, aplastic anemia, and other serious bone marrow disorders are also often acutely ill, and bone marrow examination is diagnostic. Patients with hypersplenism sufficient to cause thrombocytopenia usually have an easily palpable spleen; hypersplenism alone rarely causes a platelet count of less than 50,000/μL.

Table 22–15. Differential diagnosis of thrombocytopenic purpuras.

Thrombocytopenias due to increased platelet destruction
Immune thrombocytopenias
Idiopathic thrombocytopenic purpura
Secondary autoimmune thrombocytopenias
Drug-induced immune thrombocytopenias
Posttransfusion purpura
Neonatal immune thrombocytopenias
Thrombocytopenia due to use of factor VIII concentrate
AIDS virus infection (HTLV-III/LAV/ARV)
Consumptive thrombocytopenias
Thrombotic thrombocytopenic purpura
Hemolytic-uremic syndrome
Disseminated intravascular coagulation
Vasculitis
Sepsis
Hypersplenism
Thrombocytopenias due to decreased platelet production
Bone marrow suppression by drugs, alcohol, toxins, infections
Aplastic anemia
Leukemias and other bone marrow cancers
Megaloblastic anemia
Refractory anemias, preleukemia, hematopoietic dysplasia

Secondary causes of autoimmune thrombocytopenia, such as SLE, must be ruled out by appropriate laboratory tests. If a patient with apparent idiopathic thrombocytopenic purpura has been taking any suspicious drugs, the possibility of drug-induced thrombocytopenia must be considered. A more recent consideration in differential diagnosis is the confirmation of idiopathic thrombocytopenic purpura as part of the clinical spectrum of AIDS. In one study, 3 of 35 homosexual men with idiopathic thrombocytopenic purpura were diagnosed as having AIDS 16–34 months after their initial presentation with thrombocytopenia. It is recommended that testing for antibodies to AIDS virus (HTLV-III/LAV/ARV) be part of the assessment of idiopathic thrombocytopenic purpura.

Treatment

Splenectomy is the treatment of choice for adult patients with idiopathic thrombocytopenic purpura who have persistent symptomatic thrombocytopenia. Corticosteroids are usually able to increase the platelet count temporarily but probably do not alter the course of the underlying disease, and most patients relapse when steroids are tapered or discontinued. Adults rarely have spontaneous remissions. Splenectomy is therefore usually necessary in adults with idiopathic thrombocytopenic purpura within the first few months after diagnosis. Large doses of steroids over long periods of time should be avoided in these patients, as 75–90% will have prolonged complete remissions following splenectomy. Immunosuppressive therapy with cytotoxic drugs should generally not be used until the patient has had the benefit of splenectomy; this is

particularly true for younger patients, since these drugs may cause serious late adverse effects.

Vincristine seems to be a valuable agent in patients with autoimmune thrombocytopenia who do not respond to splenectomy, who relapse after an initial response to splenectomy, or in whom the risk of splenectomy is unacceptable. A significant increase in platelet count occurs in 70–80% of patients with refractory autoimmune thrombocytopenia treated with vincristine. Vincristine appears to be more effective, less toxic, and better tolerated than cyclophosphamide or other standard immunosuppressive drugs. Its mechanism of action in increasing the platelet count in autoimmune thrombocytopenia remains uncertain; it appears to work by a different mechanism from other immunosuppressive drugs. Other therapeutic modalities include intravenous γ-globulin.

Children with mild or moderately severe idiopathic thrombocytopenic purpura should be observed without therapy. Corticosteroids should be given when severe thrombocytopenia and bleeding occur, although the platelet count does not respond as consistently to steroids in children as in adults. Splenectomy should be considered in children only when severe thrombocytopenia persists for 3–6 months, since most children will have had a spontaneous remission by that time. The postsplenectomy state is much more likely to predispose to serious or overwhelming infection in young children than in adults. Immunosuppressive drugs should generally not be used in children.

DRUG-INDUCED IMMUNE THROMBOCYTOPENIAS

The principal drugs that may cause immune thrombocytopenic purpura are listed in Table 22–13. The best-studied example was the sedative apronalide (Sedormid) (no longer in use); the drugs most commonly used in clinical practice that can produce immune thrombocytopenic purpura are sulfonamides, thiazide diuretics, chlorpropamide, quinidine, heparin, and gold. A syndrome resembling acute drug-induced immune thrombocytopenia has also been observed in heroin addicts, although the mechanism of this kind of thrombocytopenia has not been proved. Further reports have confirmed the increasing frequency of the heparin-induced thrombosis thrombocytopenia syndrome (HITTS). This unusual combination includes clinical features of hemorrhagic tendencies due to thrombocytopenia developing in patients treated with heparin for thrombosis. It appears that the heparin-dependent IgG-class antibody induces thromboxane synthesis and aggregation of the platelets.

There is a variable period of sensitization after initial exposure to the drug, but subsequent drug reexposure is rapidly followed by thrombocytopenia. Patients therefore usually give a history of having taken the drug in the past for at least several weeks if this is their first exposure. A very small plasma concentration of the drug and very small amounts of antibody may induce severe thrombocytopenia. The drug itself generally shows only weak and reversible binding to the platelet; the thrombocytopenia in most cases appears to be caused by adsorption of the drug-antibody complexes to the platelet membrane with complement activation.

Treatment consists mainly of withdrawal of the offending drug (or all drugs) and monitoring for return of normal platelet counts, generally within 7–10 days. Thrombocytopenia may persist if the drug is excreted slowly. When a patient who is taking a number of suspicious drugs is first seen, it is often impossible to tell whether the patient has drug-induced immune thrombocytopenia or idiopathic thrombocytopenic purpura. In vitro tests can now be used in some centers to confirm drug-antibody reactions involving platelets. In vivo drug challenges of sensitized patients for confirmation of drug-induced immune thrombocytopenia should be avoided, since they are too hazardous.

POSTTRANSFUSION PURPURA

Posttransfusion purpura is an acute severe thrombocytopenic state appearing about 1 week after transfusion of a blood product. It occurs almost exclusively in women. It is mediated by an alloantibody, usually directed against the platelet PL_A^1 antigen. Platelets both with and without the PL_A^1 antigen are destroyed.

The diagnosis is suspected when acute thrombocytopenia occurs 7–10 days after blood transfusion. Coagulation studies are normal, and the bone marrow shows abundant megakaryocytes. The anti-PL_A^1 antibody is detected in the plasma.

Gradual recovery from posttransfusion purpura usually occurs in 1–6 weeks. Corticosteroids do not appear to alter the course of the disease. Massive exchange transfusions have been associated with more rapid recovery, but severe transfusion reactions often occur. Aggressive plasma exchange has also been shown to be effective without the risks of severe transfusion reactions.

IV. COAGULATION DISORDERS

HEMOPHILIA & VON WILLEBRAND'S DISEASE

Classic hemophilia and von Willebrand's disease are both congenital bleeding disorders caused by abnormalities of the factor VIII molecule complex. Hemophilia is an X-linked disorder characterized by severe deficiency of factor VIII procoagulant activity (VIII:C), which is measured in clotting assays. Von Willebrand's disease is an autosomally inherited disorder also characterized by a deficiency of VIII:C, but it is also associated with defective platelet function,

resulting in a prolonged bleeding time. The abnormal platelet function in von Willebrand's disease is due to a deficiency of factor VIII-related protein (VIIIR), which is also known as von Willebrand factor (vWF). vWF activity is measured by testing the ability of plasma to support platelet agglutination by the antibiotic ristocetin or ristocetin cofactor (VIIIR:RC) activity.

Heterogeneous antibodies made to purified factor VIII detect antigenic determinants on VIIIR (VIIIR:Ag). VIIIR:Ag has been found to be normal in patients with classic hemophilia, indicating that these patients have a normal amount of the basic factor VIII molecule but that they lack the portion of the molecule necessary for normal procoagulant activity. The heterologous antibodies therefore appear to recognize antigenic determinants distinct from the functional site responsible for procoagulant activity. Patients with von Willebrand's disease, on the other hand, have reduced levels of both VIII:C and VIIIR:Ag, indicating a true deficiency of factor VIII complex molecules. Measurement of VIII:C and VIIIR:Ag can therefore be used to differentiate between classic hemophilia and von Willebrand's disease and in most cases can differentiate between female carriers of hemophilia (heterozygotes) and normal individuals. Measurement of ristocetin cofactor (VIIIR:RC) can also be used to identify patients with von Willebrand's disease.

Human antibodies to factor VIII, unlike heterologous antibodies, are usually directed at antigenic determinants at the functional procoagulant site of factor VIII (VIII:CAg), and these antibodies are capable of blocking factor VIII clotting activity. These antibodies sometimes develop in patients with severe hemophilia after they have been transfused with factor VIII-containing blood products and sometimes develop spontaneously in otherwise healthy individuals. When present in high titer, they cause a severe hemorrhagic disorder that is difficult to correct with factor VIII transfusions, since the transfused factor VIII is simply inactivated by the factor VIII antibodies (see next section).

Some hemophiliacs treated with factor VIII concentrate have developed AIDS, and approximately 1% of confirmed AIDS patients are hemophiliacs. Some centers are switching from treating the hemophiliac with factor VIII concentrate derived from large donor pools back to treating with cryoprecipitate derived from a single donor. Infectivity can be abolished by heating the preparation.

CIRCULATING INHIBITORS OF COAGULATION

Abnormal bleeding is occasionally due to circulating inhibitors that block one or more plasma coagulation factors. These inhibitors, also called endogenous circulating anticoagulants, have in most cases been shown to be IgG antibodies. Inhibitors against factor VIII and against the prothrombin activator complex ("lupus inhibitor") occur most often, but inhibitors directed against factors V, IX, XIII, and vWF have also been reported. There are rare reports of human monoclonal proteins (especially IgM) with antibody activity directed against clotting components, eg, factor VIII, phospholipid. Inhibitors may appear abruptly and be associated with life-threatening hemorrhage or may be chronic and associated with little or no bleeding.

Factor VIII inhibitors develop in 5–20% of patients with classic hemophilia after they have been transfused with factor VIII-containing blood products; genetic factors appear to determine which patients develop inhibitors. Factor VIII inhibitors also occasionally occur spontaneously in women postpartum, in patients with autoimmune disorders such as systemic lupus erythematosus, and in older patients without demonstrable underlying disease. Rarely, the paraprotein in a monoclonal gammopathy has specific inhibitor activity against factor VIII or other clotting factors. High-titer factor VIII inhibitors (antibodies) often cause serious bleeding and require aggressive treatment. Patients with serious bleeding can be given several times the calculated amount of factor VIII to saturate the inhibitor, provided the inhibitor titer is not too high. When bleeding cannot be stopped, even after giving large amounts of factor VIII, activated prothrombin complex concentrates should be given, as these will often stop the bleeding by providing activated clotting factors which bypass the factor VIII step. If this is unsuccessful, aggressive large-volume plasma exchange can be used to remove the inhibitor. Treatment of factor VIII inhibitors with immunosuppressive drugs has also been successful in some cases.

Lupus Anticoagulant

The lupus anticoagulant was so named because of its initial identification in association with systemic lupus erythematosus, but the term has turned out to be a slight misnomer. The lupus anticoagulant should be suspected in patients with a prolongation of the partial thromboplastin time, but it is associated only with classic SLE in a minority of patients and is not associated with an in vivo hemostatic defect. Paradoxically, the lupus anticoagulant is associated with a venous and arterial thrombotic tendency. A distinct syndrome has been identified in recent years, with recurrent arterial and venous thrombosis, recurrent abortion due to placental infarction, and thrombocytopenia. Venous thrombosis may occur in unusual sites, such as hepatic, renal, retinal, and mesenteric veins. The lupus anticoagulant appears to be an IgG anticardiolipin antibody, explaining false-positive syphilis screening serologic tests. Classic SLE serologic tests in this group of patients are often negative. Specialized tests are necessary to further categorize this unusual hemostatic defect.

Recently, circulating coagulation inhibitors similar to lupus anticoagulant were reported in AIDS patients with active opportunistic infections. The inhibitors tended to disappear with successful resolution of the infection.

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23 Clinical Transplantation

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Transplantation of organ systems is becoming increasingly successful as what was once an experimental and lifesaving emergency procedure is being transformed into a life-enhancing and technologically advanced form of therapy.

The first successful renal transplant was performed at Peter Bent Brigham Hospital in 1954. Subsequently, advances in histocompatibility testing and immunosuppressive drug therapy made renal transplantation a clinical reality in the 1960s. Improved skills and handling of immunosuppressive drugs (prednisone and azathioprine) resulted in a decline in infectious complications and marked improvement in mortality rates. The 1970s witnessed the beneficial effects of blood transfusions and antilymphocyte globulin as graft-enhancing treatments. The 1980s may be characterized as the era of cyclosporine—an immunosuppressive agent that has greatly improved the success rate of kidney transplants and has also made possible heart, liver, and pancreas engraftment with better results than before. Transplant outcome has become so promising that it is now being offered early in the care of many patients with chronic and debilitating diseases.

KIDNEY TRANSPLANTATION

Patients with end-stage renal disease can be considered for renal transplantation. Absolute contraindications are conditions that would interfere with the safe administration of anesthesia or immunosuppressive therapy. These include debilitating cardiopulmonary disease, cancer, and active peptic ulcer disease or infection. Preoperative immunologic evaluation includes red cell ABO blood grouping, histocompatibility testing (determination of the patient's and potential donor's HLA antigens and the degree of haplotype matching), and state of presensitization to HLA antigens. Medical evaluation to rule out contraindications to transplantation frequently includes intestinal x-rays, voiding cystourethrography, dental and pulmonary evaluations, and assessment of cardiac status.

ABO Testing

Red cell ABO testing is performed on all recipients and potential donors. The ABO system is present not only on red blood cells but also on the vascular endothelium of the graft, with the result that renal transplants are performed only between ABO-compatible pairs. The danger of transplanting across the ABO barrier is the production of very rapid graft rejection owing to preformed isohemagglutinins that injure the vascular endothelium and elicit a coagulation reaction in situ. The same rules that apply to blood transfusion compatibility apply to renal transplantation also: for a type O recipient, the donor should be type O; for a type A recipient, the donor may be type A or O; for a type B recipient, the donor may be type B or O; and for a type AB recipient, the donor may be A, B, or O. It is possible to overcome the ABO barrier by plasmapheresis to lower the natural titer of anti-A or anti-B antibodies and by administration of cyclophosphamide to prevent new antibody formation. The long-term safety and efficacy of this experimental approach are unknown.

Living Related Donor Transplantation

All recipients and their potential donors should have complete testing for HLA-A, -B, -C, -DR, and -DQ antigens (see Chapter 6). On the basis of family typings, it is usually possible to determine the genotype or haplotype (chromosome) assignment for each identified antigen. The value of haplotype matching (0, 1, 2) was established clinically: 2-haplotype-matched siblings can be expected to achieve 90% graft survival at 1 year; 1-haplotype-matched (sibling or parent) pairs achieve 75% survival at 1 year; and 0-haplotype-matched family members achieve only 50–60% survival at 1 year.

A common clinical dilemma is the need to choose among several available compatible donors. Cellular immune assay of the mixed lymphocyte culture (see Chapter 18) is used for this purpose in several centers. Each donor who is a 1-haplotype match with the patient shares only a single set of -DR, -DQ genes. The MLC test evaluates the recipient's in vitro proliferative response to the single set of mismatched -DR, -DQ genes. One-haplotype-matched pairs with low or weak MLC responses are associated with excellent (90%) graft survival, whereas comparably matched pairs with strong MLC responses have poorer (60%) graft survival. In some centers, the low rate of graft survival among 1-haplotype-matched pairs with strong MLC responses led to the cessation of this type of transplantation until a solution was found (see Blood Transfusion, below).

Presensitization

Prior exposure to transplantation antigens can lead to sensitization manifested by the development of cy-

totoxic antibodies against HLA antigens. Patients who have antibodies to HLA antigens may have a poorer graft outcome. Moreover, patients who are sensitized and receive second and subsequent transplants are more likely to reject these grafts than those who receive a primary graft. This likelihood is especially increased in patients who rapidly rejected their first graft (< 3 months). Whether repeated rejection is caused by specific sensitization to transplantation antigens or reflects a high immune reactivity of the recipient is under investigation.

Cross-Matching

The cross-match test is used to determine the presence of any preformed antibodies (presensitization) to donor HLA antigens. A cross-match typically is performed using the patient's most recent serum and donor lymphocytes (either peripheral blood mononuclear cells or isolated T or B lymphocytes).

If the donor's cells are killed by the patient's serum, this is a positive cross-match and an indication of the presence of preformed antibodies. Positive cross-matches are a contraindication to transplantation, since they are associated with very early and uncontrollable rejection episodes leading to irreversible graft loss. The patient's own cells are also cross-matched with the same serum to reveal the presence of nonspecific autolymphocytotoxic antibodies. Such autoantibodies can cause a positive cross-match, thereby erroneously eliminating a potential donor. Special absorption of the patient's serum with self or third-party lymphocytes can frequently clarify the antibody specificity. More extensive cross-matching is done for the patient with a history of HLA antibodies by choosing additional sera previously positive with other donors or with a lymphocyte panel. While a positive cross-match against T cells eliminates a donor, positive cross-matches against B lymphocytes are only a relative contraindication, depending upon the specificity of the antibody involved. B lymphocytes not only possess HLA class I, as do T cells, but also HLA class II and surface immunoglobulin. In general, high-titered sera (> 1:8) reactive against B cells only often are considered a contraindication to transplantation, since they are usually due to anti-HLA class I antibody. Those cases in which a low-titered (< 1:8) positive B cell cross-match is obtained may be further evaluated by the use of flow cytometry (see Chapter 18). Those patients whose sera cause B cell cytotoxicity and whose antibodies are found by flow cytometry to bind to T lymphocytes may be at high risk for early graft loss.

Cadaveric Transplantation

In the circumstance where the recipient has no family members as potential donors, the opportunity exists to receive a kidney from a recently deceased individual (cadaveric transplantation). Recipients referred for this type of treatment undergo comparable immunologic evaluation of ABO grouping, HLA typing, and antibody screening and are then placed on a waiting list. While waiting, these patients often receive random blood tranfusions both to treat anemia and to increase graft survival. This exposure increases the likelihood of producing anti-HLA antibodies in response to the white blood cells or white blood cell fragments contained in each unit of blood. Other likely causes of formation of anti-HLA antibodies include pregnancy and previously rejected grafts. To monitor the extent of anti-HLA antibodies produced, serum from each recipient is collected monthly and tested in a manner known as screening. The patient's serum is cross-matched against a panel of lymphocytes obtained from many individuals. The number of individuals whose cells are killed is often expressed as a percentage of the panel (eg, 10% panel-reactive antibody). By this procedure, it is possible to determine the extent of presensitization, ie, how often the patient is likely to have a positive cross-match, assuming the transplant organ is taken from the same genetic pool of donors as the lymphocyte panel. In addition, knowing the HLA antigens on the lymphocyte panel cells that have been lysed makes it possible to analyze the specificities of the antibodies present in the serum and responsible for the positive reactions. For example, if 4 of the cells that were injured on the panel have antigen B8 in common, the antibody is considered to be anti-B8. Knowing the HLA antibody specificities in a recipient's serum allows the transplant team to avoid donors bearing those same transplant antigens. Typically, in choosing a cell panel, 40–50 cells are chosen, which allows each HLA antigen to be represented approximately twice.

Donor Selection

When a potential cadaveric donor's organs are harvested, a section of spleen, some lymph nodes, and some peripheral blood are collected. The donor ABO blood group and HLA antigens are determined from these samples. The waiting list of recipients can then be cross-matched against the donor tissues, using the patient's current serum and the recipient's highest reacting serum within the past 2 years to exclude the possibility of a positive cross-match. Those recipients who are ABO-compatible and cross-match negative become available for further consideration. Often, there may be a second round of cross-match testing among this smaller pool of recipients in which additional past sera are chosen to be certain of no hidden presensitization. From among the ABO-compatible, cross-match negative recipients, the best-matched recipients may then be selected. In programs where a large enough choice of recipients is not available to find a perfectly matched recipient, additional criteria such as length of time on the waiting list, urgency of medical condition, and whether this is a first or second transplant are considered in recipient selection.

Donor Evaluation

Family studies conducted to evaluate a potential family donor include a complete medical evaluation. There is no long-term change in survival or life-style

of individuals who have undergone uninephrectomy for donation. Because of increased success rates of related donor transplantation owing to improved immunosuppression and preoperative conditioning, it is now possible to consider living donors who share 2, 1, or 0 haplotypes with the recipient. Limited studies indicate that living unrelated donors can be considered with appropriate preoperative conditioning procedures. Evaluation of donor motivation and psychologic factors is necessary.

Cadaveric donors can be considered when determined to be neurologically dead following spontaneous intracerebral hemorrhage or head trauma. Cadaveric donors must be free from metastasizing malignancy, kidney dysfunction, or active infection (particularly with hepatitis or AIDS viruses). Hemodynamic stabilization with volume expansion and the conservative use of vasopressors maintain optimal organ function. The use of corticosteroids is controversial in cadaveric donors. The living donor nephrectomy procedure includes a flank incision through which the kidney is removed, preserving all renal arteries and veins. The ureter is removed with all periureteral soft tissue in order to include the ureteral blood supply and prevent distal ureteral avascular necrosis. Organ recovery from cadaveric donors includes removal of both kidneys with renal arteries and veins frequently left en bloc with the donor aorta and vena cava. Both ureters are removed, including all periureteral soft tissue, in order to include the ureteral blood supply and prevent distal ureteral avascular necrosis. Samples of spleen and lymph nodes are also removed for donor tissue typing and cross-matching against potential recipients.

Once removed, the kidney is flushed with mixed electrolyte solutions in order to remove all donor blood and to be cooled. Cadaveric kidneys can be stored by either of 2 methods. Cold storage involves packing in ice in order to maintain subphysiologic temperatures. Alternatively, the aorta or renal arteries are cannulated, and a cold mixed electrolyte solution is instilled by continuous cold pulsatile perfusion. Cadaver renal transplantation within the first 48 hours after donor nephrectomy is preferred.

Blood Transfusion

Recipient preconditioning can include a variety of measures. For patients awaiting cadaveric transplantation, it has been found that preoperative random blood transfusion is correlated with improved allograft survival. Untransfused patients have approximately 40–50% graft function after 2 years, whereas those patients who have received blood transfusions have a 60–80% chance of long-term cadaver graft function. Although as little as 1 unit of random blood has been correlated with improved results, the greater the number of transfusions, the better the graft survival. Mechanisms for the transfusion effect include elimination of immunologic responders who will demonstrate cytotoxic antibodies contraindicating transplantation, development of specific and nonspecific suppressor T cells, and generation of blocking or anti-idiotypic antibodies.

For individuals awaiting related donor transplantation, improved allograft success in 1-haplotype- and 0-haplotype-matched donor-recipient pairs has come through experiments involving the transfusion of donor-specific blood. These protocols call for multiple transfusions (usually 3) of small amounts of blood (100–200 mL) over several weeks during which the development of cytotoxic antidonor antibodies in the recipient is monitored. In 10–30% of recipients, cytotoxic antidonor antibodies develop, contraindicating transplantation. This antidonor sensitization can be reduced to less than 10% in the majority of 1-haplotype-matched pairs by the simultaneous administration of azathioprine (1–2 mg/kg/d) during donor-specific transfusion (DST). In the remaining 70–90% who do not become sensitized, 1-haplotype-matched allograft results have improved from approximately 65% (without DST) to 95% allograft survival after 2 years with pretransplant DST. In limited studies using 0-haplotype-matched donor-recipient pairs and unrelated living donor-recipient pairs, excellent allograft survival (> 90% at 2 years) has been achieved with DST. In 2-haplotype-matched donor-recipient pairs, random blood transfusions or DST also correlates with improved posttransplant course and allograft success (> 90% at 2 years). Mechanisms of the DST effect may include selection of immunologic nonresponders, induction of specific and nonspecific suppressor T cells, development of blocking or possibly anti-idiotypic antibodies, and clonal deletion.

Transplant Surgery

The operative procedure for the recipient includes an iliac incision through which the graft is placed in the retroperitoneal position against the psoas muscle (Fig 23–1). A renal artery anastomosis to either the internal or external iliac artery and renal vein anastomosis to the external iliac vein are standard. Ureteroneocystotomy involving anastomosis of the ureter to bladder mucosa through an anterior cystotomy incision is a usual approach. The ureter is often passed through a short submucosal tunnel in the bladder wall to prevent vesicoureteral reflux.

Postoperatively, hemodynamic stability is achieved with fluids in order to optimize renal perfusion and renal function. Careful monitoring of electrolytes, blood urea nitrogen, and creatinine to evaluate renal function is mandatory. All cadaveric kidneys have some degree of acute tubular necrosis ranging from very mild to very severe. Dialysis is required in 13–50% of cadaveric renal transplant recipients. Acute tubular necrosis is rare in patients receiving related transplants, since donor nephrectomy and recipient transplant are performed simultaneously, precluding the need for renal storage.

Postoperative Immunosuppression

Postoperative immunosuppression is the most variable aspect of recipient care. Standard immunosup-

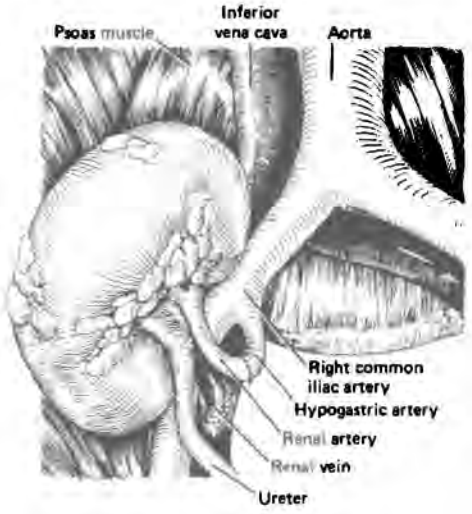

Figure 23–1. Technique of renal transplantation. (Reproduced, with permission, from Way LW (editor): *Current Surgical Diagnosis & Treatment*, 7th ed. Lange, 1985.)

pression to prevent rejection includes corticosteroids, and additional immunosuppression is chosen depending upon the type of allograft and tissue match. Cyclosporine is a fungal metabolite with immunosuppressive properties based on reduction of IL-2-mediated lymphocyte activation. The use of cyclosporine (5–15 mg/kg/d) has clearly improved long-term allograft success in recipients of cadaveric and some related transplants. Cyclosporine is definitely nephrotoxic, and monitoring of drug dosages and drug levels (100–400 μg/mL) is required.

Azathoprine, an antimetabolite that interferes with new DNA formation in proliferating cells, is frequently used as the second drug (2–5 mg/kg/d) or in combination with prednisone and cyclosporine. Azathioprine is potentially hepatotoxic, whereas cyclophosphamide is a nonhepatotoxic alternative. Antilymphocyte globulin (10–20 mg/kg) or antithymocyte globulin, a heterologous serum prepared in animals immunized with human lymphocytes or thymocytes, is a potent immunosuppressive reagent and acts through antilymphocytic properties. This heterologous animal protein can be made in horses, sheep, goats, or rabbits. Monoclonal antibodies against specific T cell subsets are also in experimental use. Lymphoplasmapheresis occasionally is used to remove recipient lymphocytes and immunoglobulin while immunosuppressive drugs are concurrently administered. Local graft irradiation has been utilized but has not provided reliable immunosuppression.

Rejection

Classic signs and symptoms of acute rejection include swelling and tenderness over the allograft and decrease in renal function. Systemic manifestations such as temperature elevation, malaise, poor appetite, and generalized myalgia can be seen. Decrease in renal function is diagnosed by a decrease in urine volume, by increasing blood urea nitrogen and creatinine levels, and radiographically by ultrasonography (blurring of corticomedullary junctions) and by radionucleotide renal scans showing decreased blood flow. In the presence of a decline in renal function, however, the differential diagnosis includes prerenal azotemia and obstruction, acute tubular necrosis, pyelonephritis, and other drug-induced toxicity. In addition, recurrence of the primary renal disease and de novo glomerulonephritis can be late causes of decreased renal function. Renal biopsy is frequently performed in order to histologically diagnose the cause of graft dysfunction.

Mechanisms of Rejection

A. Acute: Evidence suggests that blood-borne "passenger" cells (antigen-presenting cells) in grafts provide the primary stimulus. These HLA class II-positive cells are dendritic cells and (to a lesser extent) monocytes. These cells are necessary to present antigen in a form that lymphocytes can recognize. As shown in Fig 23–2, the antigen-presenting cells also provide a second signal, IL-1, which aids in triggering lymphocyte activation. IL-1 is not only involved in the activation of helper/inducer CD4 T cells but probably is important for the activation of unprimed cytotoxic CD8 T cells and B lymphocytes. The activation of helper/inducer T cells by alloantigen is pivotal to the development of cell immune responses against the graft. Once activated, these cells release IL-2, which is an essential cofactor in the activation of both CD8 T cells and B cells. As a consequence of exposure to antigen plus the interleukins, there is clonal prolifera-

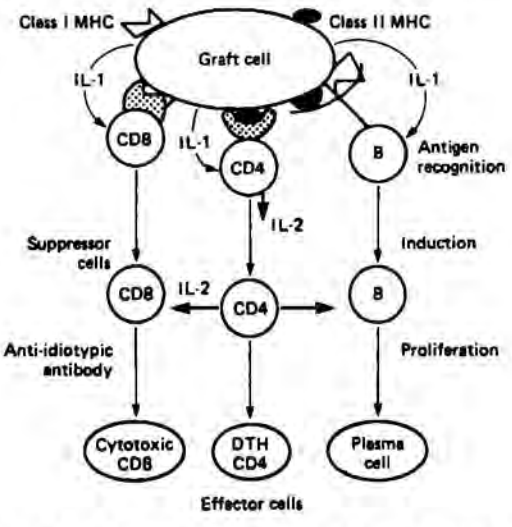

Figure 23–2. Generation of allograft rejection response (primary).

tion and maturation of alloantigen-reactive cells. This leads to the development of effector T cells, which migrate from lymphoid tissue via the blood to all tissues, including the graft, where they mediate damage at antigen-containing sites; and antibody, which is released into the blood or locally within the graft where it has access to these antigens.

The precise mechanism by which T cells destroy the graft is still under study. Effector T cells that can destroy graft tissue develop from both CD8 and CD4 subclasses (Fig 23–3). The results are similar except that CD8 T cells recognize HLA-A, -B antigen-bearing cells, whereas, CD4 T cells recognize HLA-DR antigen-bearing cells. Both CD4 and CD8 subclasses of effector cells probably can directly destroy graft cells by classic cytotoxic T cell mechanisms. However, another important consequence of T cell activation is their release of other lymphokines, especially gamma interferon (IFN γ), which can produce 2 important effects. First, IFN γ induces increased expression of HLA-A, -B, and -DR on graft tissue, which potentially makes the graft more vulnerable to effector mechanisms. Second, it activates monocytes to mediate a destructive delayed hypersensitivity response against the graft.

Hence, T cells (CD8/CD4) can directly effect target cell injury or activate macrophages into nonspecific destruction. Lymphokines in addition to IL-2 and IFN γ are released from activated T cells and include B cell growth factor and B cell differentiation factor, which play a role in directing B cell production of antibody. Antibody-mediated damage may then take place directly through complement activation or by recruitment of antibody-dependent cell-mediated cytotoxic effector cells (Fig 23–2). Most of the cells that arrive in the graft early after transplantation are lymphocytes which migrate out of the capillary and venous beds, but after 4–7 days a remarkably heterogeneous collection of cell types appears. Those of the lymphocytic series predominate over the monocyte/macrophage and include also a few PMNs. Although a variety of cell types are present, there is some evidence that early rejection of solid tissue allografts is associated with T lymphocytes having direct cytotoxic activity against donor target cells. A significant number of B lymphocytes, null cells, and monocytes also appear in the early infiltrate, and although cytotoxic T cell activity is easily demonstrated at first, later stages of rejection may involve a non-T killer cell. In all phases, the presence of antibodies and antibody-dependent cell-mediated cytotoxicity (ADCC) effector cells makes this mechanism an additional possibility. Macrophages appear to play an effector and suppressor role, while some B lymphocytes become activated and begin immunoglobulin synthesis in situ. When the host has been primed to donor antigens before transplantation, a more accelerated process, often marked by antibody-mediated vasculitis, may result.

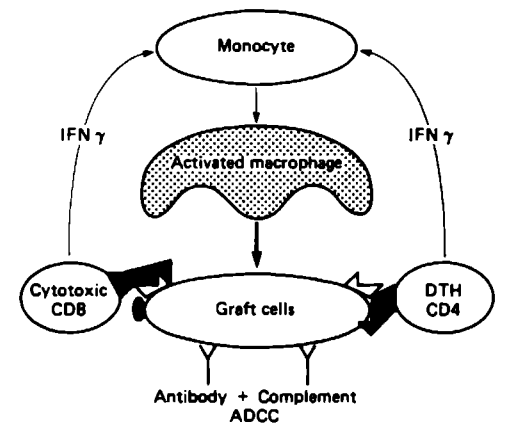

Figure 23–3. Effector mechanisms of allograft rejection.

Recent applications of anti-T cell monoclonal antibodies in staining biopsies and in vivo as therapy add considerable support to the key role of T lymphocytes in most cases of rejection. When immunofluorescence or immunoperoxidase techniques are employed with renal graft biopsies, 50–90% of the infiltrating cells are generally CD3+ and CD6+, with varying proportions of CD4+ and CD8+ cells. Although the peripheral blood often shows an increased proportion of CD4+ cells in association with acute rejection episodes, many investigators relate rejection in the kidney to a preponderance of CD8+ cells. More precisely, there is a preponderance of CD8+ cells in the blood and perivascular areas in the grafts of patients experiencing irreversible rejection (ratio of CD4+ : CD8+ < 1.0). When peripheral blood CD4+ : CD8+ ratios are higher, perivascular ratios are also higher, and rejection usually is reversible with therapy. High-dose corticosteroids, either intravenously (methylprednisolone, 1 g/d for 3 days) or orally (prednisone, 5–10 mg/kg/d for 5 days), are often used to treat acute rejection. Corticosteroids function through several pathways. They reduce the capacity of antigen-presenting cells to express class II antigens and to release IL-1. They also inhibit the alloactivation of T cells and consequently the release of IL-2. Their effect on migration and function of effector cells as well as their capacity to release IFN γ may explain their efficacy in reversing acute rejection. In this regard, they are known to produce lymphocytopenia, especially of CD4 T cells, by delaying transit of the lymphocytes through marrow and lymphoid tissues.

If there is no response or only a partial response to corticosteroids, antilymphocyte globulin (ALG) may be given (10–20 mg/kg/d for 5–14 days). ALG lyses lymphocytes, especially T cells, which makes it an excellent agent for treatment of acute rejection. ALG, however, is associated with anaphylaxis, serum sickness, and fever. Biologic effects also vary, as there is no effective measure for standardization.

Monoclonal antibodies recently have been introduced as specific therapy. The monoclonal antibody

OKT 3 (CD3) and a monoclonal antibody against activated T cells have been used to treat rejection. In the future, monoclonal antibodies against lymphokine receptors such as IL-2 or other markers of activation may be tested.

B. Hyperacute: Preformed anti-ABO isohemagglutinins or anti-HLA class I antibodies when present in sufficient quantity will bind to the vascular endothelium and trigger a cascade of immunologic events. Initially, fixation of complement components and complement activation ensues, followed by activation of the clotting pathway. This series of events, if severe enough, can result in microthrombi within glomerular capillary loops and arterioles, leading to severe ischemia and necrosis of the graft. At present, there are no effective means to treat this lesion once it begins. Emphasis is therefore placed on prevention by careful assessment of ABO blood type and donor-specific sensitization to HLA antigens by cross-match testing prior to transplantation.

C. Chronic: Chronic rejection, which can occur months to years after transplantation, is characterized by a narrowing of the vascular arterial lumen owing to growth of endothelial cells that line the vascular bed. The actual control mechanisms for this response are unknown but may include immunologic injury signals, monocyte release of IL-1, and platelet and endothelial cell release of platelet-derived growth factor. Initially, the proliferating endothelial cell lesion is reversible, but once it progresses to fibrotic changes within the blood vessel wall itself, it is unresponsive to current modes of immunosuppression and progresses to graft ischemia, extensive interstitial fibrosis, and ultimate loss of renal function. Since there is no specific therapy for this form of rejection, emphasis is again placed on minimizing chronic immunologic stimuli by seeking the greatest possible degree of histocompatibility between recipients and donors.

Outcome

Patient survival after renal transplantation is not significantly different from that of patients undergoing dialysis. In most series, patient survival at 2 years is 90–95%. Graft survival, defined as allograft function adequate to maintain life without dialytic treatment, is 75–80% at 2 years in cadaveric renal allograft recipients treated with corticosteroids and either cyclosporine or ALG. Related donor renal transplants have greater than 90% success at 2 years.

Surviving renal allografts have normal function, with mean creatinine levels of less than 2 mg/dL in most series. A functioning allograft therefore affords the recipient an optimal chance for normalization of health and minimal morbidity and mortality rates.

HEART TRANSPLANTATION

In 1967, the first successful human heart allograft was performed. Currently, the indication for cardiac allotransplantation is end-stage cardiac impairment that has been completely refractory to medical management. Absolute contraindications include severe pulmonary hypertension, infection, and cancer. Age and general medical status may represent relative contraindications. Most recipients' cardiac disease is due to coronary artery disease, cardiomyopathy, rheumatic heart disease, congenital heart disease, or benign cardiac tumors.

Cardiac donors must be individuals aged 40 or below with established neurologic death who have no preexisting cardiac disease and no significant abnormalities on chest x-ray or ECG. Hydration and conservative use of vasopressors and inotropic agents maintain optimal cardiac function and perfusion. The heart is stored by flushing the coronary circulation with mixed-electrolyte solution and preserving it on ice. The heart must be implanted within 4 hours after removal from the donor.

The cardiac transplant procedure requires that the recipient be placed on cardiopulmonary bypass. Both atria of the donor heart are anastomosed to the respective atria of the recipient, and the aorta and pulmonary arteries are likewise anastomosed. Corticosteroids, cyclosporine, and ALG are currently the preferred drugs for prevention of allograft rejection.

Pretransplant immunologic evaluation consists of HLA typing and screening for preformed anti-HLA antibodies. Although cardiac donors are routinely HLA-typed, there is rarely the opportunity to choose among several potential recipients as there is for kidney transplantation. Hence, the potential benefits of HLA matching have not yet been realized. Nevertheless, careful cross-match testing is routinely performed, and only cross-match negative recipients are chosen. In addition, donors bearing specific HLA antigens against which the recipient has made corresponding antibodies are avoided.

The diagnosis of allograft rejection is made on the basis of endomyocardial biopsy performed through a transvenous catheter placed in the right jugular vein. A mononuclear cell infiltrate is the characteristic hallmark of rejection. Lymphocytes, lymphoblasts, and monocytes are the predominant cell types seen in acute rejection. Evidence of tissue damage is found in necrosis of myocardial fibers and edema, which further impairs perfusion and function. Electrocardiographic changes of decreased voltage, sometimes associated with arrhythmias and signs of congestive heart failure, can also be demonstrated during rejection episodes. Treatment of rejection includes the use of ALG and increasing levels of corticosteroids. Chronic rejection can also occur and is usually associated with atherosclerotic changes of coronary vessels. Successful retransplantation has been offered to individuals with chronic allograft rejection. Currently, graft survival at 1 year is greater than 60% in most series. For patients transplanted during the last decade, survival at 5 years is approximately 40%. Patients enjoy excellent ventricular function, and over 60% are able to return to employment or satisfactory rehabilitation.

Combined Heart-Lung Transplantation

Transplantation of solitary lungs has had poor results, frequently owing to lack of healing at the bronchial anastomosis, and thus at present heart-lung transplantation is the procedure of choice for patients with pulmonary hypertension. The procedure requires anastomosis of the trachea, right atrium, and aorta. Lymphatic circulation to the lungs is reestablished within several weeks after operation. Although the lungs remain denervated, this does not appear to contribute to ventilatory failure. Blood supply to the trachea is provided through collaterals from the coronary circulation. Currently, immunosuppression includes the use of cyclosporine, azathioprine, and antithymyocyte globulin (ATG) in the early postoperative course, and corticosteroids after establishment of tracheal healing. The diagnosis of rejection is made by endomyocardial biopsy, since cardiac rejection usually occurs simultaneously with lung rejection. Although pure lung rejection can occur, diagnosis is made indirectly through changes on chest x-ray. Survival of heart-lung transplants after 1 year is approximately 70%. Patients with successful heart-lung transplantation can enjoy excellent rehabilitation.

LIVER TRANSPLANTATION

Experimental liver transplantation has been performed in animals for over 30 years. The first human liver transplant was performed in 1963 by Starzl, and since then more than 800 orthotopic liver transplants have been performed around the world.

Liver grafts have been thought to be "immunologically privileged." In pigs and rats, allogeneic kidney and heart grafts done without immunosuppression are rejected in 7–21 days, while liver allografts between the same strains have prolonged or indefinite survival. Moreover, after liver grafting, the animals tolerate subsequent skin grafts from the same donor for prolonged periods, but promptly reject third-party skin grafts. Prolonged acceptance of liver grafts in rats is strain-dependent because certain strains express different levels of immune responsiveness to transplantation antigens of the donor. In addition, in certain strain combinations, the liver graft appears to reduce recipients' alloreactivity. Thoracic duct lymphocytes from long-term liver-grafted rats are specifically deficient in those clones effecting graft rejection, and when serum from these animals is infused into other rats of the same recipient strain, the new rats accept third-party allografts for prolonged periods. Unfortunately, immunosuppressive factors have not been found in similar experiments on dogs or primates, and so there have been no applications of these findings to clinical human transplantation.

Indications

The decision to perform liver replacement involves a judgment concerning the clinical status of the patient and the donor organ supply. Hepatic dysfunction is manifested by alterations in both synthetic and regulatory ability, and patients should not have severe systemic complications of liver disease before transplantation. At present, many potential liver recipients die before a suitable organ becomes available.

The largest number of adult patients who have received a liver transplant suffered from hepatitis B antigen-negative postnecrotic cirrhosis or chronic active hepatitis. Some patients with primary hepatocellular tumors have received transplants, and a few have been cured, but in over 60% the tumor has recurred. Despite administration of hepatitis B immune globulin during the anhepatic phase, transplant patients with hepatitis B antigen-positive chronic active hepatitis continued to have antigenemia, and a high percentage eventually succumbed to recurrent hepatitis. Liver transplantation in either of these types of patients may not be warranted unless transplantation is combined with other systemic therapeutic modalities.

Infants and children with congenital or developmental anomalies of the bile ducts are the customary pediatric recipients of liver transplants. Extrahepatic biliary atresia is the most common cause of bile duct obstruction in infants, and a liver transplant is often considered after failure of a hepatoportal enterostomy (Kasai procedure). Other indications for liver replacement in children are inborn errors of metabolism such as α_1-antitrypsin deficiency, tyrosinemia, and Wilson's disease. As outcome has improved, liver replacement has been used to treat liver-based inborn errors of metabolism that result in extrahepatic organ system failure; eg, one patient with homozygous familial hypercholesterolemia received a heart-liver transplant and one with oxalosis received a liver-kidney transplant.

Procedure

Orthotopic transplantation, the most commonly employed method, entails removal of the host liver and its replacement in the right upper quadrant with a cadaver allograft. **Heterotopic transplantation** entails placement of a liver at an ectopic site. This method has not met with much success but has been used when temporary hepatic failure has occurred. In orthotopic transplantation, the donor organ is retrieved as the recipient hepatectomy is begun. The donor liver is flushed with cold solutions and placed in hypothermic storage but must be used within 6–10 hours. The recipient hepatectomy is the most technically difficult phase of the operation because many of these patients have portal hypertension and previous surgery. There may be excessive bleeding and numerous adhesions at the operative site. The anhepatic phase is the time of greatest physiologic stress because the portal vein and venae cavae have been clamped, and there is decreased venous return to the heart. The revascularization phase requires attention to hemostasis. The suprahepatic inferior vena cava is anastomosed to the recipient vena cava, and the donor portal vein is anastomosed to the recipient portal vein in an end-to-end fashion. The infrahepatic vena cava is sewn to the

donor inferior vena cava, and finally the donor celiac artery is sewn to the recipient common hepatic artery. The bile duct is usually sewn to the recipient common bile duct, but when this is not possible, the bile duct is sewn to a Roux-en-Y loop of jejunum. Improvements in surgical technique, new technologic advancements such as the venovenous bypass system, and the ability to control coagulation have decreased operative mortality rates.

Outcome

Early failure is related to the quality of preservation of the donor liver and to the development of infection. In the event of severe ischemic damage occurring during either donor maintenance and harvesting or preservation, the only alternative is immediate retransplantation. Survival after infection is related to the type of offending organism. Bacterial infections from pulmonary, bladder, or vascular sites usually respond to antibiotics. Infections caused by fungal or viral organisms are often related to extent and severity of immunosuppression and are more difficult to treat. Late failure is due to rejection or recurrence of the original disease. The survival rate at 1 year is 68% for all patients who have received a liver transplant and 74% for children.

Cross-Matching & Immunosuppression

In liver transplantation, decisions regarding suitability of an organ are currently based solely on ABO blood type and organ size and not on HLA antigen matching. Many liver transplants are performed in children, and the donor liver must be of an appropriate size to fit into a child's abdominal cavity. There are only a few reports of hyperacute rejection when the liver is transplanted across a positive cross-match, but transplantation is not usually done in recipients who demonstrate preformed antibody. Why the liver apparently is resistant to hyperacute rejection is not known. Hyperacute rejection is presumed to be due to the presence of preformed antiendothelial antibodies, and because of the unique vascular architecture of the liver, these antibodies may have little effect. In some studies, decreased levels of cytotoxic antibodies were noted early after revascularization. There was no demonstration of increased antibody fixation to the graft, but the loss of the cytotoxic antibodies could have been due to the formation of soluble immune complexes.

When rejection occurs, it is a diagnosis of exclusion. There is no single diagnostic test. Biliary obstruction, viral hepatitis, drug toxicity, and recurrence of underlying disease must be excluded before rejection is diagnosed with certainty. The signs and symptoms of rejection include fever, upper abdominal pain, and decreased appetite. Ascites and hepatomegaly are found, and laboratory abnormalities include elevations of the serum bilirubin, prothrombin time, alkaline phosphatase, and transaminase values. Liver scans usually show poor concentration of the radionuclides. A liver biopsy is then performed. Three histopathologic features suggest rejection when all occur together: a mixed cellular portal inflammatory infiltrate, bile duct damage, and central or portal vein endothelial inflammation. This triad is observed in acute rejection and in graft-versus-host disease but is not seen in other forms of liver disease. Treatment for rejection consists of a bolus of 500–1000 mg of methylprednisolone.

Before cyclosporine was available, only a 30–40% 1-year survival rate was achieved with azathioprine and corticosteroids. The survival rate of liver transplant recipients treated after 1979 with low-dose corticosteroids and cyclosporine is 70–75% after 1 year. At the time of liver implantation, prednisone is administered and subsequently decreased during the postoperative and convalescentperiod to a baseline dose of 20 mg. At some centers, cyclosporine is administered preoperatively, 2 mg/ kg/d intravenously, and is increased to 6 mg/kg/d postoperatively. When oral intake begins, cyclosporine is administered at a dose of 15 mg/kg/d and then adjusted based on blood levels. Bile formation is necessary for absorption of cyclosporine, and in a poorly working graft, inadequate absorption may necessitate intravenous administration. While hepatotoxicity has been reported with the use of cyclosporine, elevated postoperative liver function test values are usually due to rejection. These considerations are unique to the use of cyclosporine, the drug which has been the single greatest contribution to the success of liver transplantation. The search continues for more selective immunosuppressive agents and better ways to control allograft rejection.

PANCREAS TRANSPLANTATION

Unlike liver transplantation, which is often a life-saving procedure, pancreas transplantation can be considered only a life-enhancing procedure at present. Pancreas grafts can be whole or segmental, or dispersed islets of Langerhans can be used, to provide biologically responsive insulin-producing tissue. The purpose of these procedures is to improve the quality of life of the diabetic patient and to prevent the vascular changes associated with diabetes mellitus. Most uremic diabetic patients improve considerably with a kidney transplant, but the other long-term complications of diabetes—retinopathy, angiopathy, and neuropathy—do not improve. Better metabolic control may prevent the occurrence of or halt the progression of secondary systemic complications, but perfect metabolic control cannot be attained by conventional insulin administration techniques. This situation, along with the hypothesis that the most physiologic approach to maintaining euglycemia in the diabetic is with a pancreas transplant, has provided the impetus to develop a successful procedure. Initially, islet cell transplantation was thought to be safer and simpler than other alternatives, but it remains experimental.

Clearly, there is an autoimmune component to the pathogenesis of insulin-dependent diabetes mellitus

type I (IDDM) (see Chapter 31). A mononuclear cell infiltrate surrounding the islets of Langerhans has been detected at the time of diagnosis of IDDM (insulitis), and autoantibodies directed against islet cytoplasmic and cell surface antigens are found in the sera of type I diabetics. There is a strong association of IDDM with other organ-specific autoimmune endocrinopathies, and there is an association with the HLA-DR3 and -DR4 alleles. Careful examination of the sera (and in one instance immunohistologic study of the pancreas) has demonstrated that the majority of infiltrating lymphocytes are of the T cytotoxic/suppressor phenotype. This histologic picture resembles that seen in other autoimmune diseases and in allograft rejection. Therefore, distinguishing recurrent autoimmune disease from rejection may prove difficult in transplanted pancreatic tissue. It is expected that transplantation will serve as an excellent probe of the pathogenesis of diabetes.

Over 500 pancreas transplants have been performed throughout the world, and over 40 living related donor transplants have been performed at one center. Six of the latter grafts failed for technical reasons. Of the technically successful allografts, the survival rate at 1 year was 95% and the functional graft survival rate 41%. As may be expected, higher graft survival rates are associated with transplants between HLA-identical siblings and pancreas transplants from donors who previously had given a kidney to the recipient. However, the role of autoimmune responses assumes greater importance under these circumstances. Insulitis has recurred in 3 living related transplants between identical twins and in 7 transplants from related donors that resulted in graft failure. These findings have necessitated changes in the immunosuppression of living related recipients, and in general, enthusiasm about these types of transplants has waned.

Indications

Ideally, pancreas transplantation should be performed before the patient has developed severe secondary diabetic complications. It will be difficult to balance the risks of long-term immunosuppression with the risks of developing the systemic complications of diabetes. Commonly, pancreas transplants have been performed on patients who were uremic and had already received a renal transplant. However, some centers now perform pancreas transplants on patients who have neither uremia nor kidney transplants but have other progressive secondary complications that outweigh the risks of long-term immunosuppression.

Procedure

The pancreas is removed from the donor and preserved in cold storage. It can be transported from a distant retrieval site to the facility where the transplant will take place if the preservation time does not exceed 6 hours. Simultaneous implantation of the kidney and pancreas from the same donor in the same recipient is no longer frequently performed, because the longer operative procedure results in higher patient morbidity than a sequential kidney-pancreas transplant and demonstrates no benefit of increased pancreas graft survival.

Various surgical techniques are used for implanting a pancreas graft. The grafts are implanted either as a whole organ with a small button of donor duodenum or as the distal segment of the pancreas. Living related transplants can be performed only with segmental grafts. For segmental grafts, the body and tail making up about 50% of the pancreas are removed and the donor vessels connected to the iliac vessels of the recipient. Another option with segmental grafts is to implant the celiac and portal vessels of the donor to the splenic vessels of the recipient, so that the venous effluent will drain into the portal circulation. While delivery into the portal system is physiologic, there is no convincing evidence that systemic delivery is inferior. The pancreatic duct is either occluded by injecting a synthetic polymer or left open and connected to the gut or the urinary system. Better long-term graft function has been achieved with open-duct management, but there is no difference in graft function between urinary drainage or drainage into a jejunal loop.

Outcome

The major complications are infection, development of ascites, pancreatitis, vascular thrombosis, preservation injury, and rejection. Overall graft function and patient survival rates have improved steadily since the first clinical pancreas transplant was performed in 1967. The present overall graft survival rate at 1 year is 40%, and the patient survival rate is 77%. With the adoption of whole-organ transplantation and urinary drainage in small series of patients, 1-year graft survival rates of 70% are being reported.

Cross-Matching & Immunosuppression

Pancreas transplant donors and recipients are typed and matched for the ABO and HLA groups, and a transplant is not performed across a positive crossmatch. The exact role of tissue matching has not been clearly defined. In those patients who have received a simultaneous pancreas-kidney transplant, both organs are not uniformly rejected. In some instances, the pancreas graft has failed (presumably from rejection) while the kidney graft continues to function. In those instances where the pancreas and kidney are from different donors, there again is no rejection of one organ over the other that can be attributed to the differences in tissue type alone. With living related transplants, the benefit of HLA matching was evident, as 76% of the grafts from HLA-identical siblings, 58% of the grafts from mismatched relatives, and 30% of the grafts from cadaver donors were functioning at one year. Nevertheless, some centers never use tissue matching as a criterion for cadaver pancreas transplantation.

Rejection in the vascularized pancreatic allograft is recognized by loss of control over blood glucose levels—a relatively insensitive and late finding. Disap-

pearance of insulin from the circulation usually parallels the plasma glucose level. Corticosteroids used for immunosuppression also tend to cause diabetogenic effects. Hyperglycemia may be associated with a decrease in serum C-peptide levels. Serum amylase levels have not been useful in the diagnosis of rejection, but in those transplants drained into the urinary system, low levels of urinary amylase are suggestive of graft failure. This finding usually precedes by 24–48 hours the changes in blood glucose levels; therefore, antirejection therapy can be instituted earlier. Current practice at some centers is to perform a biopsy of the graft when the question of rejection occurs, but even then a diagnosis is not assured. The presence of vasculitis is the only sure sign of rejection, because parenchymal fibrosis and inflammatory cell infiltrates may be secondary to a foreign body reaction, especially in duct-injected grafts or recurrent disease.

Successful immunosuppression for pancreas transplantation is more difficult to achieve than for kidney or liver transplants. In patients who previously had received a kidney transplant and were immunosuppressed at the time of pancreas implantation and for HLA identical sibling transplants the use of cyclosporine and corticosteroids has been satisfactory.

In patients who are not uremic or who are not kidney transplant recipients, a stronger immunosuppressive protocol, (cyclosporine, azathioprine, and prednisone together) has been used.

Rejection usually is treated with bolus intravenous corticosteroids, a temporary increase in the oral prednisone dose, or a temporary course of ALG. A role for ALG, irradiation, or monoclonal antibodies has not been clearly determined in any clinical setting.

Islet Cell Transplantation

The successful placement of isolated pancreatic islets in nonidentical donors has been pursued for a long time. It was hoped that unmodified islets might display prolonged survival similar to other endocrine tissues. However, isolated islet allograft survival is shorter than allografts of skin, kidney, or heart no matter where they are placed. Syngeneic grafts of pancreatic islets implanted in the liver or placed under the renal capsule produce insulin and are able to reverse hyperglycemia in virally induced diabetes, diabetes caused by β-cell toxins, and spontaneously developing diabetes in BB rats and NOD mice. Since autografts cannot be used in most clinical situations, the major thrust has been to perfect allograft islet transplantation.

Islets cells are obtained from fetal and adult pancreases. The fetal pancreas contains less connective tissue, and so the yield of viable islets is greater but still not high enough to render one recipient euglycemic. The basic method for retrieving islets is mechanical separation by various means and then enzymatic digestion, usually with collagenase, of the crude tissue. Secondary steps of separation are then required to remove as much nonislet tissue as possible. This often necessitates handpicking of the tissue. The purer the islet preparation is, the longer the graft survives. However, the islets still contain dendritic cells and cells capable of stimulating an immune response. Various investigators have tried different means to reduce the immunogenicity of this tissue. Treatment of the islets with an anti-HLA class II (DR) monoclonal antibody or irradiation has met with some success. Culturing fetal pro-islets for prolonged periods of time in an oxygen-rich atmosphere has also been successful in reducing tissue immunogenicity. An alternative method that has been successful in rats and mice involves the encapsulation of individual islets within a semipermeable biologic membrane.

All of these experiments have been performed in animal models. Prolonged reversal of the diabetic state has been achieved using modified islet allografts without concomitant immunosuppression. However, graft failure occurs in long-term survivors because the autoimmune process recurs. Therefore, immunosuppression will most probably be necessary even with islet allografts.

BONE MARROW TRANSPLANTATION

Modern clinical bone marrow transplantation began in earnest in 1968 when a small number of patients with advanced leukemia, severe combined immunodeficiency disease (SCID), and Wiskott-Aldrich syndrome received marrow infusions from HLA-identical siblings. Prior observations in animals had shown that matching donor and recipient at the MHC reduced the incidence of graft-versus-host (GVH) disease and improved survival rates. Many patients have now survived for more than a decade after bone marrow transplantation for a variety of malignant and nonmalignant hematologic diseases. Laboratory and clinical advances in such areas as histocompatibility typing, prevention of GVH disease, improved supportive care, and reduced risk of relapse have made bone marrow transplantation a realistic and successful form of transplantation for usually fatal diseases (Table 23–1).

Until recently, most donors for bone marrow transplantation have been either identical twins (syngeneic) or genotypically HLA-identical siblings (allogeneic). Only 25% of patients can be expected to have an HLA-identical donor, and efforts to use marrow from partially matched family members and phenotypically matched unrelated donors are beginning to be successful. For those diseases not involving the bone marrow, autologous transplantation allows the use of high-dose chemoradiotherapy and avoids the risk of GVH disease. Provocative studies using monoclonal antibodies to leukemic and other malignant cells have given credence to the idea that such marrow "purging" techniques could greatly extend the concept of autologous transplants to those patients assumed to have indiscernible neoplastic cells in the marrow.

The 3 major categories of diseases treatable by bone marrow transplantation are **severe combined immunodeficiency disease (SCID), aplastic ane-**

Table 23–1. Diseases treatable by bone marrow transplantation.

Syngeneic/Allogeneic	Autologous
Aplastic anemia	Leukemia
Leukemia	ALL
AML	CML
ALL	Lymphoma
CML	T cell
Lymphoma	Burkitt's
T cell	Other
Burkitt's	Solid Tumors
Immunodeficiencies	Testicular
Common variable	Ovarian
SCID	Neuroblastoma
Wiskott-Aldrich syndrome	Lung (small cell)
Agranulocytosis (Kostmann's syndrome)	Head and neck
	Breast
Osteopetrosis/genetic diseases	
Solid tumors	

mia, and leukemia. The cure of other genetic diseases by bone marrow transplantation is feasible (Table 23–2), but controversy remains about relative risks and benefits.

Bone marrow transplantation is the treatment of choice for children with congenital SCID and variants. For HLA-matched transplants, no immunosuppressive conditioning is necessary. Partially matched recipients require conditioning—usually with cyclophosphamide and busulfan rather than radiotherapy. The removal of T cells from donor marrow by lectin agglutination or monoclonal antibody and complement lysis enables parents of haploidentical children with these disorders to serve as donors.

Aplastic anemia has a mortality rate of 90% when treated with supportive care. Allogeneic bone marrow transplantation increases survival to 45% overall and to 70% below age 30. Furthermore, if patients are able to avoid pretransplantation transfusions—and thus presensitization—overall survival increases to 75%. Unlike the case for leukemia, rejection of the marrow in aplastic anemia has been a major cause of failure, and this is probably due to the underlying autoimmune nature of aplasia in some patients, to presensitization by transfusions, and to the lack of radiotherapy in the conditioning regimen. Irradiation is given to kill leukemic cells but also contributes greatly to immunosuppression. Omitting irradiation results in fewer complications and less toxicity but increases risk of rejection. However, despite decreased rejection, irradiation shows no benefit in survival, because of increased complications. For patients over age 30, ATG may be more effective initially, and bone marrow transplantation should be reserved for those patients not responding to ATG.

Table 23–2. Bone marrow transplantation for genetic diseases.

Severe combined immunodeficiency
Wiskott-Aldrich syndrome
Fanconi's anemia
Kostmann's syndrome
Chronic granulomatous disease
Osteopetrosis
Ataxia-telangiectasia
Diamond-Blackfan syndrome
Mucocutaneous candidiasis
Chédiak-Higashi syndrome
Cartilage-hair hypoplasia
Mucopolysaccharidosis
Gaucher's disease
Thalassemia major

Acute myelogenous leukemia has a mortality rate of over 90% in adults. The success of allogeneic bone marrow transplantation in this disease has been confirmed by studies worldwide, including 3 randomized trials of bone marrow transplantation versus chemotherapy. In the initial trials for relapsed acute leukemia, a long-term relapse-free survival rate of 13% was achieved in patients with no hope of survival by any other means. When patients receive allogeneic transplants while in remission from acute nonlymphocytic (myelogenous) leukemia (ANLL), relapse rates are 15–20%, and overall survival is 40–75%, depending on the age of the recipient. Data strongly indicate that allogeneic bone marrow transplantation will provide 60–80% relapse-free survival in patients with chronic myelogenous leukemia (CML). More favorable results are produced when the transplant is performed soon after diagnosis and when patients are below age 30. Especially compelling are cytogenetic analyses confirming the absence of the Philadelphia chromosome as long as 5 years after transplantation. Results in patients with acute lymphoblastic leukemia (ALL) have not been as favorable, mainly due to the higher posttransplantation relapse rate of 50%. Changes in the conditioning regimen to optimize treatment without adding to toxicity are needed.

Procedure

Unlike other organ transplants, bone marrow aspirated from the iliac crests of a donor is entirely regenerated in 8 weeks. Since the amount harvested is only 20% of the total, the donor is not harmed immunologically or hematologically. Multiple aspirations of 5 mL each, yielding a total of about 10 mL/kg of the recipient's weight (600–1000 mL), are obtained under general or epidural anesthesia. The marrow is drawn through heparinized needles and placed into heparinized, buffered culture medium. This mixture is then gently filtered through fine stainless steel mesh screens to produce a single cell suspension. Nucleated cell counts are checked to ensure the adequacy of the withdrawn marrow. If the donor and recipient are ABO-compatible, $2–6 \times 10^8$ marrow cells per kilogram are infused intravenously together with red cells (red cell volume of 20–30%). If donor and recipient are not ABO-compatible, either the recipient must undergo plasmapheresis to remove the anti-A or anti-B isoantibodies or the red cells must be removed from

the donor's marrow in vitro. Unlike the case for renal transplantation, donor-specific pretransplant transfusions do not seem beneficial, probably because most of the recipients have been heavily isoimmunized by prior transfusions and also because the immune system is totally ablated prior to transplantation.

Except in patients with SCID, destruction of the recipient's immune system is necessary to prevent rejection and to allow transplantation of an entire hematopoietic system including new immunocompetent cells. This is usually accomplished by giving cyclophosphamide, 50–60 mg/kg for 4 or 2 days (the higher dose for those patients not receiving total body irradiation). The dose of total body irradiation is 7.5–15 Gy, which is often administered in fractions over 3–5 days rather than in a single dose, to avoid toxicity to the lungs and eyes. This combination of chemotherapy and radiotherapy provides a potent immunoablative and antineoplastic function for most cancer patients.

Following preparative chemoradiotherapy and infusion of the marrow, patients are extremely vulnerable to infections and bleeding. Strict isolation in rooms with laminar air flow has been shown to have a significant effect on outcome only for patients with aplastic anemia. Simple precautions such as filtering the air to remove airborne fungi and hand-washing are important. The early and aggressive use of broad-spectrum antibacterial antibiotics (semisynthetic penicillins or cephalosporins and aminoglycosides), as well as acyclovir and amphotericin B, is critical. Accepted practice dictates that these antibiotics not be discontinued until absolute neutrophil counts are above 500/μL following engraftment. The role of trimethoprim-sulfamethoxsazole in preventing *Pneumocystis carinii* pneumonia is clearly established. The use of intravenous immunoglobulins is logical and attractive but not yet proved. Granulocyte transfusions are not given prophylactically at most centers because of the lack of evidence that they are beneficial and because of risks of secondary infections, notably cytomegalovirus pneumonia. Platelet transfusions, on the other hand, are given to keep the platelet count above 15,000/μL to prevent serious spontaneous hemorrhage. All blood products must be irradiated to prevent GVH disease from viable lymphocytes in transfused cellular components or plasma.

Engraftment is heralded by a rising white cell count, relative monocytosis, and the appearance of circulating mature neutrophils 2–4 weeks after transplantation. Bone marrow samples at 2 and 4 weeks show increasing cellularity, and the platelet and reticulocyte counts also begin to rise. In general, all hematopoietic and immune cells of the recipient are replaced by donor cells, although there are rare examples of mixed "chimerism," most often in children who receive transplants for immunodeficiency diseases. As peripheral counts improve, antibiotics can be discontinued and transfusions become unnecessary. Patients can be discharged when they can be followed closely as outpatients, twice weekly to daily for at least the first 100 days after transplantation.

Posttransplantation Complications

The major obstacles to successful bone marrow transplantation are GVH disease, infections, interstitial pneumonia, veno-occlusive liver disease, and relapse of the underlying disease. GVH disease and infections are responsible for 10–30% of morbidity and mortality in the first 30 days following transplantation.

Graft-versus-Host (GVH) Disease

The presence of immunocompetent donor cells in an immunocompromised host is a prerequisite for GVH disease. Host and donor are histoincompatible. In patients who are HLA-identical with their donors, the occurrence of GVH disease is attributed to "minor," presently undetectable differences in histocompatibility. GVH disease also can develop from lymphocytes in random blood tranfusions given to neonates, patients with congenital immunodeficiencies, and cancer patients who are immunocompromised either by virtue of their disease (eg, T cell leukemias, Hodgkin's disease) or chemotherapy.

The clinical syndrome of GVH disease in humans consists of skin rash, severe diarrhea, and jaundice. Pathophysiologically, immunocompetent T cells (CD8 suppressor; T8 or Leu 2 phenotype) can be found in biopsies of the skin, intestine, and liver. These tissues appear to be especially at risk because they are rich in surface DR antigens. The skin rash of acute GVH disease usually begins at the time of engraftment, 10–28 days after transplantation. It is a fine, diffuse, erythematous, macular rash often beginning on the palms, soles, or head and spreading to involve the entire trunk and sometimes the extremities. In severe GVH disease, the rash can become desquamative—the clinical equivalent of an extensive second-degree burn. Watery diarrhea is associated with malabsorption, cramps, and gastrointestinal bleeding when severe. Hyperbilirubinemia is due to inflammation of small bile ducts caused by GVH disease, and it is usually accompanied by an elevated serum alkaline phosphatase level. Elevations of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) are mild to moderate. A staging and grading system for GVH disease developed at the University of Washington has become standard (Table 23–3). Acute GVH disease occasionally is delayed until 30–70 days after transplantation, and then it is almost always the harbinger of chronic GVH disease.

Successful prevention of acute GVH disease began with the use of methotrexate after transplantation to outbred DLA-matched dogs. Its use in humans by the Seattle Bone Marrow Transplant Center has remained the standard by which other measures to prevent GVH disease are judged. Immunosuppressive therapy with methotrexate, cyclophosphamide, or cyclosporine is given for the first 3–12 months after transplantation. Nevertheless, approximately 50% of patients develop acute GVH disease within 10–70 days after grafting, and up to half may die. Infusions of antihuman thymocyte globulin, prednisone, cyclosporine, and in vivo monoclonal antibodies have been used to treat estab-

Table 23–3. Clinical stage of GVH disease according to organ system.

Stage	Skin	Liver	Intestinal Tract
+	Maculopapular rash <25% body surface	Bilirubin 2–3 mg/dL	>500 mL diarrhea/d
++	Maculopapular rash 25–50% body surface	Bilirubin 3–6 mg/dL	>1000 mL diarrhea/d
+++	Generalized erythroderma	Bilirubin 6–15 mg/dL	>1500 mL diarrhea/d
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/dL	Severe abdominal pain with or without ileus

lished acute GVH disease with limited success. Incubating the donor marrow in vitro with anti-T cell monoclonal antibodies plus complement or similar antibodies coupled to toxins, or using a soybean lectin agglutination and SRBC rosette forming technique has successfully depleted the marrow of T cells and lowered the incidence of GVH disease. Interestingly, strict isolation in rooms with laminar air flow also has decreased the incidence of acute GVH disease, but only in patients with aplastic anemia. Current trials using a combination of immunosuppressive drugs are encouraging in further decreasing the incidence of GVH disease. Unfortunately, studies of patients receiving T cell-depleted marrow have shown an increased risk of rejection, higher relapse rate of leukemia, increased risk of fungal infections, and risk of posttransplant EBV-related lymphoproliferative disease. These findings support the concept that donor T cells have an active graft-versus-leukemia effect and that they may also provide protection against fungi. A more precise dissection of T cell varieties might solve this problem. Another way to use the beneficial anti-GVH disease effect of T cell depletion without complications might be to detect specific activation antigens on those cells mediating GVH disease and not on those mediating graft-versus-leukemia. A third solution might be less complete ("imperfect") T cell depletion.

Chronic GVH disease affects 25–45% of patients surviving longer than 180 days. It occurs more frequently in older patients and those with preceding acute GVH disease. Clinically, it most resembles the spectrum of collagen-vascular or autoimmune disorders, and its main clinical effect is to produce severe immunodeficiency leading to recurrent and life-threatening infections, much like those seen in the congenital and acquired immunodeficiency syndromes (AIDS). Treatment with prednisone, alone or in combination with azathioprine, can effectively reverse many of the manifestations of chronic GVH disease in 50–75% of affected patients.

Infections

Infectious complications following bone marrow transplantation are due to the profound lack of granulocytes and lymphocytes following ablation by the pretransplant conditioning regimen. Since full recovery of these 2 major elements of the immune system occurs separately following transplantation, it is not surprising that the risk of infection can be separated into 3 distinct phases.

The first and riskiest phase is the 2- to 4-week period immediately following infusion of the marrow, when no circulating leukocytes are present. During this time, patients are at risk for both bacterial and fungal infections, which can advance extremely rapidly and cause death. Clinical experience over the past 15 years has led to the aggressive, empiric use of broad-spectrum antibiotics (both antibacterial and antifungal). Coverage must be begun at the first sign of infection, such as fever, chills, localized pain, or change in mental status. Waiting for the results of cultures often results in overwhelming and irreversible sepsis or pneumonia. The spectrum of organisms causing infections has gradually changed. Infections caused by gram-positive organisms were usually treated successfully in the early days of bone marrow transplantation. The recent rise in infections due to resistant species of staphylococci, especially *Staphylococcus epidermidis* responsive only to vancomycin, is most likely due to the use of central intravenous catheters in these patients. The use of these catheters has been a major factor in improved support, and there are no viable alternatives. As a result, vancomycin is empirically added to the antibiotic regimen when fever persists. Sepsis and pneumonia due to gram-negative cocci have long been known to be rapidly fatal in patients with profound granulocytopenia. Marrow transplant patients are no exception. Powerful, synergistic combinations of antibiotics (semisynthetic penicillins or advanced cephalosporins and aminoglycosides) have greatly diminished the number of deaths due to these organisms. Nevertheless, there remains a high risk of death owing to bacterial infection—largely from gram-negative organisms—during the period of marrow aplasia following transplantation. The overall risk is much higher when acute GVH disease is present, ranging from 10% to 40% depending on age and underlying disease. Future efforts are aimed at the use of intravenous antibiotics before infections arise, intravenous immunoglobulins, and modified reverse isolation techniques.

Two to four weeks after transplantation, the marrow begins to export granulocytes successfully to the blood; when the absolute granulocyte count reaches 500/μL and is rising, the greatest threat of bacterial infection is past. The second phase of potential infec-

tious complications is due to immaturity of the lymphocytes, and the greatest risk is due to fungal and viral agents during the second and third posttransplant months. An especially prominent pathogen is *Aspergillus fumigatus*, which can cause vascular invasion in the lungs and brain. Although these infections can be treated with amphotericin B, they are difficult to eradicate and often are fatal. The most prominent viral pathogen is cytomegalovirus (CMV), which has a mortality rate of 80% when it causes pneumonia. Treatment has been ineffective to date, although newer, more promising antiviral drugs are being developed.

Interstitial pneumonia due to nonbacterial pathogens, notably CMV, fungi, and noninfectious agents, remains a major complication of bone marrow transplantation. The incidence of interstitial pneumonia is about 35%, with a case fatality rate of 70–85% and an overall mortality rate of 24%. In about half of cases, no cause can be found, and these idiopathic pneumonias are considered to be secondary to the toxicity of chemoradiotherapy. Risk factors for the development of interstitial pneumonia are (1) older age of the recipient, (2) use of methotrexate to prevent GVH disease, (3) grade of GVH disease, (4) pretransplant performance status, (5) interval from diagnosis to transplant, and (6) dose-rate of total body irradiation.

The third period of infectious risk occurs after the third month and lasts until the maturation of the lymphocytic arm of the immune system. This parallels the neonatal period and takes 6–18 months. During this time, there is an abnormal ratio of helper to suppressor T cells; T cells function poorly in response to antigens; and immunoglobulin production is abnormal. This leads to a risk of infection by encapsulated bacteria such as pneumococcus because of a lack of opsonic immunoglobulins. The higher risk of viral infection diminishes as T cell function gradually improves. Patients must continue to remain relatively isolated until the immune system has fully recovered. Those patients who have chronic GVH disease may never totally recover full normalcy of the immune system. However, the majority of surviving patients do recover full immunity and lead lives free from infection, requiring no antibiotics or other supplements.

BONE TRANSPLANTATION

Bone is more commonly transplanted than any other tissue. In general, bone grafting operations are performed to promote healing of un-united fractures, to restore structural integrity of the skeleton, and to facilitate cosmetic repair. Human skull defects over 2–3 cm large are closed by neurosurgeons to protect the brain and restore bony integrity. Plastic surgeons, oral surgeons, and periodontists use fresh autografts and freeze-dried allografts in oral and maxillofacial surgery. Various bone grafts are used to promote stability of the spine and correct spinal deformity. Autografts and allografts are used for repair of the appendicular skeleton (arms and legs). Where autograft sources are insufficient, allogeneic bone may be used but only in combination with an autograft, which provides a greater degree of early repair. Procurement of bone for implantation is by aseptic removal or removal and subsequent sterilization by ethylene oxide or gamma irradiation. Except for a fresh autograft, all other bone tissues are used after freezing because of the reduction in immunogenicity achieved by this storage technique.

Postransplantation Course

Following grafting one of 3 courses can occur: the bone graft may become viable, acquiring the mechanical, cosmetic, and biologic characteristics of adjacent bone; it may partially or completely resorb without satisfactory new bone formation, leaving disfigurement or instability; or it may become sequestrated, encapsulated, and treated by the host as a foreign body. The most likely graft to achieve optimal function in humans is the fresh autograft. However, allogeneic implants are becoming more widely used.

A bone graft transferred to a recipient undergoes several adaptive phases before ultimate incorporation into the skeletal system. Osteogenesis from surviving cells of the graft itself is characteristic only of fresh autografts. By contrast, cells from an allograft usually elicit antibody production and cell-mediated immunity and start to decay. These alloimplants slowly revascularize by invasion of capillary sprouts from the host bed during the process of resorption of the old matrix. Finally, in both autografts and allografts, osteoinduction occurs by the process of recruitment of mesenchyme-type cells into cartilage and bone under the influence of a diffusible **bone morphogenetic protein** derived from the bone matrix. Bone morphogenetic protein is a recently discovered glycoprotein with a molecular weight of 17,500. The target cell for its activity is an undifferentiated, perivascular mesenchymal cell whose protein synthesis is reprogrammed in favor of new bone formation.

Temporally, healing of bone grafts follows a well-known pattern. For the initial 2 weeks, an inflammatory response occurs associated with infiltration of the graft by vascular buds and the presence of fibrous granulation tissue, osteoclast activity, and osteocyte autolysis. There occurs a "creeping substitution" of graft bone manifested as mesenchymal cells differentiating into osteoblasts that deposit osteoid over devitalized trabeculae. Dead trabeculae are later remodeled internally. Thus, through appositional new bone formation, the graft is strengthened. In contrast to cancellous bone, cortical bone grafts undergo a somewhat longer period of resorption and slower appositional phases of new bone formation. This results in only half strength being acquired during the first 6 months and full strength 1–2 years after grafting.

Immunologic Rejection

Since bone is a composite of cells, collagen, ground substance, and inorganic minerals, all but the minerals are potentially immunogenic. Cell surface

transplantation antigens associated with the MHC are the most potent immunogens within osteochondral allografts and are found on cells of osteogenic, chondrogenic, fibrous, neuronal, fatty, hematopoietic, and mesenchymal origin. Cell-rich marrow contributes significantly to immunogenicity.

Fresh allogeneic bone can sensitize the host and cause the production of circulating antibodies. Nevertheless, cellular immunity is thought to be more important than humoral antibodies in causing rejection of allogeneic bone transplants. Cartilage seems to resist destruction by antibody and cellular resorptive mechanisms, but if an immune response by the recipient develops, this protection is only relative, and a low-grade, slow, immunologically mediated inflammatory response ensues, characterized by an increase in synovial fluid, white cell counts, antibody response, and pannus reactions.

Rejection of allogeneic bone (cortical or cancellous) elicits a response that delays healing at the site of osteosynthesis and blocks revascularization, resorption, and appositional new bone formation. Clear-cut rejection or failure of the graft occurs in only about 10% of bone grafts.

Immunosuppression

Temporary systemic immunosuppression has been used, since MHC antigens are present in bone for only 2–3 months after transplantation. Drugs that have successfully allowed bone union include azathioprine, corticosteroids, cyclosporine, and cyclophosphamide. Because of side effects and the low rate of graft failure, these agents are no longer routinely used in human musculoskeletal transplantation. A promising new technique to diminish the antigenicity of grafts is the use of a temporary biodegradable cement that coats the donor bone and hides the bone cell antigens until these cells have died and their MHC antigens have deteriorated.

Clinical Recovery

Early ambulation and mild exercise stimulate blood flow and osteogenesis within the graft. External splinting helps to stabilize the graft. Education of the patient in proper posture, weight bearing, turning, and exercise has been helpful in allowing sufficient time for healing.

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Bone Transplantation

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Allergic Diseases

24

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The allergic diseases are a diverse group of conditions characterized by immunologically induced inflammation in which the antigen (allergen) comes from the environment. Exposure to the allergen may be through inhalation, ingestion, skin contact, or injection. The presence of an allergic disease indicates that a prior exposure to the allergen has induced an immune response (sensitization). The clinical features of allergy (sensitivity, hypersensitivity) are those of the host's inflammatory response to the presence of allergen and are not dependent on the chemical nature of the allergen. The allergic reaction has epitope (antigenic determinant) specificity.

Allergic diseases may be generalized, or they may be localized to a particular organ or tissue. Target tissues show varying degrees of inflammatory cell infiltrate, alterations in vascular tone and permeability, and changes in visceral smooth muscle and glandular activity.

Types of Allergic Reactions

Allergic diseases have diverse pathogenetic processes, reflecting the diversity of the immune response. The widely used classification scheme of Gell and Coombs, based on immunologic pathogenesis, is outlined below.

Type I: IgE antibodies cause disease through involvement of mast cells that have specific IgE surface receptors. Exposure to allergen activates the mast cell to release or generate chemical mediators with vasoactive and inflammatory properties. Histamine, leukotrienes (formerly known as slow-reacting substance of anaphylaxis [SRS-A]), and eosinophil chemotactic factors of anaphylaxis (ECF-A) produce immediate effects that are evident within minutes after exposure to an allergen. Neutrophil chemotactic factors (NCF) are responsible for a late phase inflammatory response that occurs after several hours. Platelet activating factor (PAF), serotonin, and kinins may also be involved. The allergen-IgE antibody-mast cell-mediator mechanism is responsible for the atopic diseases, anaphylaxis, and urticaria.

Type II: IgG or IgM antibodies (or both) activate complement through the classic pathway. Under appropriate conditions, antigen and antibody localized on circulating erythrocytes, leukocytes, or platelets cause drug-induced antibody-dependent lysis of these blood cells. (See discussion of drug allergy, below.)

Type III: IgG or IgM antibodies form circulating immune complexes with antigen and complement, activating complement-derived chemotactic factors and producing localized tissue inflammation. This mechanism is responsible for the Arthus reaction and serum sickness. Hypersensitivity pneumonitis in its acute form may be a type III alveolitis (see Chapter 26).

Type IV: Sensitized T lymphocytes react with allergen, thereby generating lymphokines. This mechanism is involved in allergic contact dermatitis (see Chapter 29).

Other immunologic pathways are potentially capable of producing allergic inflammation, although none have been clearly identified with a particular clinical disease. The alternative pathway of the complement system can be activated by IgA antibodies. Activation of complement through either the classic or alternative pathways generates anaphylatoxins (C3a, C5a, C4a) that can release mediators from mast cells, thus inducing reactions similar to those associated with type I allergy. There is experimental evidence that IgG4 subclass antibodies sensitize skin mast cells for wheal-and-erythema responses.

Prevalence

The prevalence of allergic diseases varies with the type of allergy and with the population at risk. Allergic contact dermatitis probably affects 30% or more of the population. The atopic diseases produce symptomatic illness in about 10% of Americans. Serum sickness induced by therapeutic injections of large amounts of antiserum derived from animals occurs in about 90% of those so treated. Penicillin hypersensitivity occurs in about 3% of the population, although anaphylaxis to penicillin is rare. Allergy to most other drugs is uncommon.

Susceptibility to Allergy

Allergy affects only a portion of the exposed population. The occurrence of allergic disease depends upon factors of susceptibility to sensitization by allergen and other factors that determine target organ localization and clinical expression of the disease. The antigenic specificity and intensity of the immune response and the immunoglobulin class of the antibody are under genetic control, but genes controlling target organ localization have not been identified. A viral respiratory infection occurring coincidentally with exposure to allergen may exert an adjuvant effect on sensitization. The quantity and route of exposure to allergen can affect both the sensitization to allergen and the provocation of allergic symptoms.

Nonallergic Diseases

Each of the diseases in which allergic reactions are expressed—eg, asthma, rhinitis, atopic dermatitis, contact dermatitis, anaphylaxis, urticaria-angioedema, and drug-induced cytolysis—can also arise through nonimmunologic means. This is clinically important in differential diagnosis. In some cases the illness proceeds in the absence of an external trigger, such as in chronic nonallegic rhinitis, nonallergic asthma, idiopathic urticaria, and idiopathic anaphylaxis. In other cases, an environmental agent can activate inflammatory mediators nonimmunologically; examples are nonspecific histamine release by opiates (a direct mast cell effect), asthma from aspirin (possibly an aberrant metabolism of arachidonic acid), anaphylactoid reactions from iodinated radiocontrast media, urticaria from shellfish and berries, and asthma from inhalation of isocyanates. In these examples, no allergen-specific immunologic sensitivity has been identified even though only a small portion of the exposed population is at risk.

GENERAL CONSIDERATIONS IN DIAGNOSIS

The diagnostic process is aimed at determining whether the patients's disease is caused by allergy—and, if so, at identifying the specific allergen or allergens responsible. Simple cases of seasonal hay fever caused by pollen or contact dermatitis caused by poison ivy may be correctly diagnosed with minimal effort, but more complex or obscure allergic diseases require considerable detective work.

History

A thorough history is essential. A complete description of the symptoms will often distinguish allergic from nonallergic conditions. Variations in symptoms during the course of a day, week, month, and year and the association with home, work, school, or vacation trips are useful clues in diagnosis of the common inhalant and occupational allergies. The allergy environmental history should include details of work, hobbies, pets, drug use, and dietary habits; the influence of weather and climate on respiratory symptoms; and the effect of contactants such as plants, perfumes, cosmetics, clothing, and topical medications in suspected allergic dermatoses. The course of the illness, other known allergies, family history of allergy, and the effect of prior treatments provide additional diagnostic information.

Physical Examination

The results of physical examination should be correlated with the current history of allergen exposure, since allergic manifestations occur only in the presence of allergen. A complete physical examination and appropriate follow-up examinations are usually indicated in diagnosis.

Laboratory Testing

Selection of laboratory tests should be based on clues to suspected allergic mechanisms and to the most likely allergens derived from the results of the history and physical examination. Testing blood, respiratory secretions, and stools for eosinophils may be useful in some cases of type I allergy. X-rays and pulmonary function tests should be ordered as indicated.

A. Antibody Tests: Determining the specific allergens causing disease requires identification of a specific antibody or of specific T cell sensitivity. However, these tests prove only that exposure and an immune response to the allergenic epitope has occurred. This information must be interpreted in light of the history to determine its clinical relevance. Thus, a positive skin test or in vitro test for sensitivity to a specific allergen indicates only a potential state of hypersensitivity.

1. Skin testing for IgE antibodies—Skin testing is the method generally used to confirm sensitivity in patients with atopic disease or anaphylaxis. Within minutes after introduction of the allergen, histamine released from skin mast cells causes vasodilatation (erythema), localized edema from increased vascular permeability (wheal), and pruritus. The skin reacts to allergen in almost all patients with type I allergy, even though their disease occurs in the nasal mucosa, conjunctiva, bronchi, or gastrointestinal tract. Many tests with different allergens can be performed simultaneously. Skin testing is convenient, safe, and reliable, and experience over many years has shown it to be useful for diagnosis in most patients with suspected allergic disease if care is taken to correlate the findings with the history and other clinical information.

Antihistaminic drugs inhibit or diminish skin test responses and must be discontinued 24 hours or more before testing. Hydroxyzine is inhibitory for as long as 1 week. Xanthines, sympathomimetic drugs, corticosteroids, and cromolyn sodium do not inhibit immediate skin test reactions and need not be withdrawn prior to testing.

Best results are obtained by using a combination of cutaneous and intracutaneous methods, and each test series should include the diluent as a control. Some allergists also include histamine or a nonspecific histamine liberator (or both) as positive controls, although these are not necessary for routine use.

a. Cutaneous tests—Cutaneous tests by either the prick or scratch method should always be done first. The tests are applied to the back or to the volar surfaces of the forearms, depending upon the number of tests. In prick testing, the skin directly under a drop of concentrated allergen extract is pricked with a needle. After 20 minutes, the drop is wiped off and the reaction is quantitated and recorded as indicated in Table 24–1. When the test is properly done, the control is negative and a 2+ or greater result is significant. Allergens giving a negative or 1+ prick test should be retested intracutaneously.

Scratch tests are done by making a short linear scratch in the skin to which the allergen is then ap-

Table 24–1. Wheal-and-erythema skin tests.

	Reaction	Appearances
Prick	Neg	No wheal or erythema.
	1+	No wheal; erythema < 20 mm in diameter.
	2+	No wheal; erythema > 20 mm in diameter.
	3+	Wheal and erythema.
	4+	Wheal with pseudopods; erythema.
Intracutaneous	Neg	Same as control.
	1+	Wheal twice as large as control; erythema < 20 mm in diameter.
	2+	Wheal twice as large as control; erythema > 20 mm in diameter.
	3+	Wheal 3 times as large as control; erythema.
	4+	Wheal with pseudopods; erythema.

plied. This method is more likely to produce nonspecific irritant reactions, causes more discomfort to the patient, and occasionally leaves scars.

b. Intracutaneous tests—Negative or questionable cutaneous tests are repeated by the more sensitive intracutaneous method, which should be done only on the extremities, preferably the lateral aspect of the upper arm or the volar portion of the forearm. No more than 0.01 mL (preferably 0.005 mL) of sterile extract is injected intracutaneously, and the reaction is read in 20 minutes as indicated in Table 24–1. A 2+ or greater reaction is considered positive. Some allergens give false-positive irritant reactions if a high concentration is injected, so the proper concentration is important and must be determined for each allergen. A 1:500 (w/v) dilution of pollens and fungi is generally satisfactory for routine use. Some allergists use serial dilution titrations for each allergen, but this is time-consuming and rarely provides more diagnostic information than a single properly selected dilution, except in testing for Hymenoptera insect venom anaphylaxis.

After the immediate wheal and erythema subside, a late-phase 6- to 12-hour reaction of diffuse induration appears in some cases. The diagnostic significance of the late-phase skin reaction is currently unknown.

2. In vitro tests for IgE antibody—IgE antibodies in serum can be detected and semiquantitatively measured by the radioallergosorbent test (RAST), enzyme-linked immunosorbent assay (ELISA), or similar serologic methods (see Chapter 17), avoiding the potential risk or discomfort of skin testing. These tests are applicable only to those allergens that can be chemically coupled to the immunosorbent, and they are less sensitive than skin testing, since tissue mast cell-fixed IgE antibodies can exist in the absence of circulating antibody. Furthermore, results are influenced by high levels of total serum IgE and by IgG blocking antibody in patients who have received immunotherapy.

Most allergists rely on skin testing as the primary diagnostic procedure because of ease of administration, availability of results in 20 minutes, low cost, and extensive experience with clinical correlation. An in vitro test might be preferable in cases of dermographism, extensive inflammatory skin disease, inability to eliminate antihistamines prior to testing, or in some infants or small children.

3. Skin testing for IgG antibodies—The Arthus reaction has been used as a diagnostic test for IgG antibodies. It is elicited by injecting 0.1 mL of sterilized allergen extract intradermally and reading at 5–8 hours. A positive test consists of induration, usually with tenderness and erythema. This method of testing is not in common use but has been reported to be helpful in some cases of occupational hypersensitivity pneumonitis caused by organic dusts. A preliminary prick test should be done, and the test should be withheld if the patient has a high degree of coincidental IgE sensitivity to the allergen.

4. In vitro test for IgG antibodies—The precipitin-in-gel test is simple to perform, but it is positive only in the presence of large amounts of circulating antibody. More sensitive tests are ELISA and RAST, modified to detect IgG antibodies.

5. Tests for T lymphocyte cellular hypersensitivity—

a. The tuberculin skin test (see Chapter 17) is a well-established indicator of cellular sensitivity. It is performed by injecting 0.1 mL of test solution intradermally and reading at 48 hours; 10 mm or more of induration constitutes a positive test.

b. The patch test is the standard technique for detecting cellular sensitivity in allergic contact dermatitis. An appropriate dilution of the test substance is applied to the skin and left open ("open patch test") or covered with a taped patch ("closed patch test"). A positive result consists of erythema, papules, or vesicles present at 48 hours. A photoallergic contact reaction requires exposure to ultraviolet light or sunlight after the patch is removed ("photo patch test"). The patch test requires a concentration of test substance below the limit of nonspecific skin irritation. A standard set of patch test reagents is available from the American Academy of Dermatology for the most common agents causing allergic contact dermatitis, and the standard textbooks on contact dermatitis list proper concentrations for testing hundreds of other chemicals.

c. In vitro tests for cellular sensitivity are based on changes in lymphocyte morphology, stimulation of cell division, and release of lymphokines in the presence of allergen (see Chapter 18).

B. Provocation Testing: In some instances, it is desirable to demonstrate sensitivity of a particular target organ on exposure to the allergen in vivo under controlled conditions. A positive provocation test does not prove that allergy is the cause of the reaction. However, the nature of the clinical reaction provoked and the time course and threshold concentration of a positive reaction, combined with the clinical history and results of immunologic tests, are useful in assessing the role of allergy in the diagnosis.

1. Bronchoprovocation—An aqueous aerosolized extract of allergen is inhaled through a nebuliz-

ing device in increasing concentrations. In cases of suspected asthma, a pulmonary function test—usually forced expiratory volume in 1 second (FEV_1)—is performed at intervals to detect an immediate response (approximately 30 minutes), a late response (approximately 6 hours), or dual (immediate and late) responses. Recurrent severe nocturnal asthma has been described after a single bronchial challenge with allergen.

The procedure has a number of drawbacks. Aqueous allergen extract in aerosol form is not deposited in the same portion of the airway as are naturally inhaled pollen grains, mold spores, or other allergenic particles. Some extracts may be irritating and therefore may give nonspecific responses. The testing procedure is cumbersome and time-consuming compared to skin testing. A positive bronchial challenge may provoke severe bronchospasm, causing discomfort and danger to the patient, so the procedure must be done in a hospital for 24-hour observation of the patient with appropriate measures for immediate control of the severe asthmatic reaction. The test is not practical for routine evaluation but may be useful for definitive testing of occupational allergens.

2. Nasal provocation—The bronchial technique can be adapted for nasal inhalation, but techniques for measuring nasal airway obstruction are less well standardized.

3. Oral food challenge—Foods to be tested are eliminated from the patient's diet prior to the test. Freeze-dried food extract is packed into opaque capsules, and weighed amounts are fed orally in increasing doses. Up to 8 g of food can be delivered in a single challenge by this method. Some foods can be disguised in flavored milkshakes. Testing is done in a double-blind fashion, using placebo controls. Urticaria and gastrointestinal symptoms occurring within 2 hours are the usual positive responses to double-blind oral food challenges. Patients with suspected anaphylaxis to food should never be tested by this method. A negative double-blind food challenge should be followed by open trial of the food in the diet for final confirmation.

ALLERGENS

Any foreign substance capable of producing an immune response is a potential allergen. Some are more likely to be allergenic than others, and each type of allergic disease is associated with certain common environmental allergens. A wide variety of chemical structures have been shown to be allergenic. Complex organic natural chemicals, especially proteins, are likely to cause antibody-mediated allergy, whereas simple organic chemicals and inorganic compounds and metals are more frequently a cause of cell-mediated allergy.

For diagnostic purposes, allergens are most conveniently classified by route of exposure as inhalants, ingestants, or injectants. Contact allergy is covered in Chapter 29.

Inhalants

Plant pollens, fungal spores, animal danders, and certain airborne particles in the home are the most common inhalant allergens for IgE-mediated type I allergic rhinitis and asthma.

Wind-pollinated (anemophilous) plants discharge large numbers of lightweight buoyant pollen grains into the air that can be dispersed by wind currents over a wide area. Within each geographic location, the common allergenic trees, grasses, and weeds pollinate each year during a specific and predictable season. For example, in the eastern and midwestern USA, the important allergenic trees—maple, elm, oak, and birch—pollinate for 6–8 weeks beginning with the spring thaw; grass pollen appears principally during June and July; and the weeds pollinate from the middle of August until the first frost. Pollinating seasons in the far west are long and overlapping. In the San Francisco Bay Area, for example, there are about 12 important allergenic trees with pollen seasons covering the period from December through September; this overlaps with the grass and weed pollen season, which begins in April and continues through October (Table 24–2). Allergists must be familiar with the allergenic plants and pollinating seasons in their area. Air-sampling devices for identifying and quantitating pollen are available, but their use requires knowledge of pollen morphology.

Plants with attractive flowers are generally insect-pollinated, producing small amounts of heavy pollen that do not become airborne and are thus not usually the cause of inhalant allergy.

Spores of fungi in soil and on decaying vegetation are important aeroallergens and are found in air samples in significant quantities throughout the year except when there is snow cover on the ground. Al-

Table 24–2. Allergenic plant pollens and mold spores in the San Francisco Bay Area.*

Trees and Shrubs	Weeds	Grasses	Fungi
Acacia	Beach sandbur	Bermuda	*Alternaria*
Alder	Cocklebur	Bluegrass	*Aspergillus*
Ash	English plantain	Brome	*Cephalothecium*
Birch	Lamb's quarters	Orchard	*Fusarium*
Box elder	Mugwort	Perennial rye	*Helminthosporium*
Cottonwood	Pickleweed	Sweet vernal	*Hormodendrum*
Cypress	Pigweed	Velvet	*Mucor*
Elm	Ragweed, false	Wild oat	*Penicillium*
Juniper	Ragweed, western		*Rhizopus*
Live oak	Russian thistle		
Mulberry	Sheep sorrel		
Olive	Wingscale		
Privet			
Sycamore			
Walnut			

*Example of one area's allergenic pollens. For further details regarding other geographic areas, see Sämter M, Durham OC: *Regional Allergy of the US, Canada, Mexico and Cuba.* Thomas, 1955; and Roth A: *Allergy in the World.* Univ Press of Hawaii, 1978.

though sensitivity to fungi is less common than pollen allergy, the spores of *Alternaria, Hormodendrum, Helminthosporium, Aspergillus, Pullularia, Mucor, Rhizopus, Penicillium,* and other fungi are important allergens for some patients with asthma. Their role in causing allergic rhinitis is less certain. Rusts and smuts that infect certain crops and grasses also produce allergenic spores.

The spores of thermophilic actinomycetes are very small and are probably capable of being inhaled into distal airways, where they are more likely to cause hypersensitivity pneumonitis than asthma.

In certain localities, insect debris has been identified as the cause of allergic respiratory symptoms.

House dust is the most common indoor allergen. For many dust-sensitive patients, the allergen is a house dust mite, *Dermatophagoides farinae* or *Dermatophagoides pteronyssinus*. These mites flourish on human skin scales and are found especially in dust from pillows and mattresses. Feathers in down pillows, quilts, comforters, sleeping bags, or jackets may be allergenic. Danders or excretions from household pets (cats, dogs, hamsters, guinea pigs) or from horses, farm stock, or zoo animals also cause allergy.

Occupational respiratory disease can be caused by allergy to organic chemicals such as phthalic anhydride, trimellitic anhydride, and antibiotics; inorganic compounds such as chloroplatinates and nickel salts; and dust particles of proteins such as enzymes and grain dusts.

Ingestants

Foods, drugs, and food and drug additives may be allergenic.

A. Foods: Atopic food allergy is most often caused by the food protein or by a product of partial digestion. Carbohydrates, fats, additives such as preservatives or flavoring and coloring agents, and contaminating drugs are other potential allergens in foodstuffs. Closely related foods may contain common or cross-reacting allergens; for example, some patients react to all legumes, including beans, peas, and peanuts. Some food allergens are heat-labile, so that sufficient cooking may render the food nonallergenic. Less commonly, the reaction occurs to the cooked food only.

Based on double-blind food challenges, legumes, milk, eggs, fish, and nuts are the most common causes of allergic reactions. These are IgE-mediated, occur within 2 hours of ingestion, and usually cause gastrointestinal symptoms and pruritic skin lesions.

Thousands of natural and artificial additives are used to preserve and enhance the quality of foods sold commercially. Allergy to food additives is frequently suspected but rarely confirmed. Metabisulfites used as antioxidants in many foods may produce sulfur dioxide when the food is ingested, thereby provoking asthmatic attacks in some asthmatic patients through an unidentified mechanism which is probably not immunologic.

Allergy to foods tends to diminish with age. Infants and children with food sensitivities frequently tolerate these foods without difficulty later in life. It is not known whether this is an acquired immunologic tolerance or the consequence of physiologic maturation of the digestive process, preventing absorption of intact food proteins.

B. Drugs: Drugs cause a variety of allergic reactions, usually functioning as haptens to bind covalently with a host carrier protein. In some cases, a drug metabolite is the allergenic hapten. Protein drugs such as sera, vaccines, biologicals, and allergen extracts are antigenic per se and carry a high risk of inducing allergic sensitization.

Many factors influence the allergic potential of a drug. Topical administration is more likely to induce sensitization than are the oral or parenteral routes. The presence of an active infection may increase the risk of drug sensitization. Atopy and other immunologic diseases have not been shown to predispose to drug allergy. Certain patients are multiple drug reactors, possibly on a genetic basis. Children are less susceptible than adults. Allergy to a particular drug is independent of its pharmacologic properties but dependent rather upon the ability of the drug or its metabolite to bind covalently to carrier protein.

Type I allergic reactions to drugs include anaphylaxis, urticaria, and angioedema. These can occur with any drug, but systemic anaphylaxis is most likely to result if the drug is given by injection. Severe reactions and even death, however, have occurred from oral administration of penicillin.

Type II allergic reactions are complement-dependent and therefore involve IgG or IgM antibodies. The drug-antibody-complement complex is fixed to a target cell, usually a circulating blood cell, resulting in complement-dependent cell lysis. Such reactions may involve erythrocytes, leukocytes, or platelets.

There are 4 mechanisms by which drugs can induce immunologic damage to cells: (1) The drug first fixes to the cell membrane, followed by reaction of the antibody to the cell-fixed drug antigen, resulting in a cell-antigen-antibody complex. The complex then activates the complement sequence, with lysis of the cell. Immunohemolytic anemia from penicillin is an example of this mechanism. (2) The drug-antibody-complement complex is formed first in the plasma, and the complex secondarily fixes to the cell, after which lysis occurs. In these reactions, the direct antiglobulin (Coombs) test is positive. Autoimmune hemolytic anemia, leukopenia, and thrombocytopenia from quinidine, sulfonamides, and stibophen are examples of this type of drug reaction. (3) The membrane of red blood cells may be modified by drugs so that the cells adsorb circulating immunoglobulins nonspecifically to give positive antiglobulin tests. However, disease from this mechanism is rare. (4) Methyldopa causes hemolytic anemia by inducing autoantibody formation. The drug affects the red cell membrane, exposing erythrocyte autoantigens that induce an autoimmune hemolytic anemia that can persist even after the drug has been withdrawn.

It is possible that some drugs may produce type II reactions by more than one of these 4 mechanisms.

Type III drug reactions are exemplified by serum sickness, a term applicable to the reaction whether caused by heterologous serum or by a haptenic drug such as penicillin. The disease is a multisystem complement-dependent vasculitis in which immune complexes are deposited along the endothelial surfaces of blood vessels, stimulating inflammation and vascular wall damage. There is a latent period of several days after administration of the drug before sufficient antibody is produced to generate immune complexes capable of activating the complement system.

Type IV (cell-mediated) allergy is the mechanism resulting in allergic contact dermatitis from topically applied drugs. Topical antibiotics, antihistamines, local anesthetics, and certain additives found in topical medications, including parabens and lanolin, are frequent causes of this type of allergy.

In many adverse drug reactions, allergy is strongly suspected but the immunologic mechanism is difficult to prove. Allergy is suggested by (1) a reaction occurring in a small proportion of persons exposed to the drug; (2) a latent period between exposure to the drug and the appearance of a reaction, which is of shorter duration with succeeding exposures to the drug; (3) elicitation of the reaction by very small doses; and (4) an association with other signs suggesting allergic disease, such as eosinophilia. Erythematous, morbilliform, or other skin eruptions, drug fever, cholestatic liver disease induced by certain drugs, and drug-induced interstitial nephritis belong to the category of suspected allergic reactions.

Certain drug reactions can be caused by allergic or nonallergic means. Urticaria and angioedema may be produced either by type I allergy to a drug or by nonspecific liberation of mast cell histamine by drugs such as morphine.

An allergic reaction to a drug occurs after exposure sufficient to induce an immune response, so that some patients may react even though the drug has been used frequently in the past without incident. A reaction on first exposure suggests prior sensitization by a cross-reacting drug or antigen. Once a reaction occurs, any subsequent use of the drug, sometimes even in trace amounts, can cause a recurrence of symptoms.

Penicillin can cause almost every known type of allergic reaction. Anaphylaxis occurs about once in every 10,000 patient courses of the drug and accounts for about 300 deaths in the USA each year. Urticaria or angioedema appearing within an hour after administration of the drug is a form of anaphylaxis. Urticaria that begins days to weeks after the drug is administered has less serious implications and in some cases may disappear even if penicillin treatment is continued. The most common manifestation of penicillin allergy is a diffuse erythematous or morbilliform skin eruption. Serum sickness occurs occasionally, and immunohemolytic anemia may complicate high-dosage intravenous therapy. Allergic contact dermatitis is common after topical penicillin. It is estimated that some type of adverse reation to penicillin occurs in 3% of patient courses of the drug.

Penicillin toxicity is exceedingly low, so the appearance of an adverse reaction during therapy almost always indicates allergy to the drug. An erroneous diagnosis of penicillin allergy is frequently applied to cases of exanthematous eruptions complicating infections treated with penicillin. This is often the case when ampicillin is used for treatment of infectious mononucleosis, other viral infections, and streptococcal diseases.

Injectants

Injected drugs and diagnostic reagents may cause anaphylaxis or urticaria-angioedema. (See discussion of drugs above.)

The sting of a hymenopteran results in injection of venom that contains protein allergens in addition to the pharmacologically active chemicals responsible for the usual localized inflammation. In honeybee (family Apidae) venom, the major allergen is phospholipase A, and minor allergens are hyaluronidase and melittin. In vespids (family Vespidae), which include hornets, yellow jackets, and wasps, the venom allergens have not yet been identified but are different from those found in the honeybee. IgE antibodies to these allergens are responsible for systemic anaphylaxis.

GENERAL CONSIDERATIONS IN TREATMENT

A state of potential hypersensitivity exists when an immune response occurs, but the hypersensitivity remains asymptomatic and does not require treatment until there is exposure to the allergen. Prophylactic treatment by avoidance of allergens is usually the most effective means of treatment. However, avoidance is not always possible in practice, necessitating the use of medications to control symptoms. In some cases, the immune response itself can be altered by immunotherapy.

Environmental Measures

The ideal treatment in allergy is to avoid the allergen, and in the case of drug and food allergy this is usually the only available option. Avoidance of an allergen is based on proof in the clinical history of symptomatic allergy and not on a positive skin test alone. Appropriate measures in individual cases may be elimination of household pets, control of house dust exposure by frequent cleaning and avoidance of dust-collecting toys or other objects in the bedroom, and dehumidification and repair of leaking pipes or roofs to prevent mold growth. Avoidance of pollen and outdoor molds is not possible unless the patient is able to stay in an air-conditioned home or office. In some cases, the patient might arrange a vacation trip to a pollen-free area during the peak pollen season.

In cases of occupational allergy, every effort should be made to modify the patient's job and employ

industrial hygiene measures before considering a change in the patient's work.

Pharmacotherapy

Many drugs are helpful for control of allergic symptoms, and only those most frequently used will be discussed here.

A. Antihistamines: Since histamine is only one of several endogenous mediators of type I IgE allergic reactions, antihistamines may have a limited effect on ameliorating symptoms. Furthermore, the pathophysiology of histamine involves stimulation of 2 classes of target cell receptors. H_1 receptors are involved in allergy, and they mediate increased vascular permeability, vasodilatation, itching, and bronchial and gastrointestinal smooth muscle contraction. H_2 receptor stimulation is involved in gastric acid secretion and possibly in some T cell functions.

H_1 receptor antagonists are reversible competitive inhibitors for the H_1 receptor and therefore block histamine effects if administered before exposure to allergen. Several chemical classes of compounds are represented in the large number of drugs currently available (Table 24–3). The drugs are most useful in treatment of allergic rhinitis and in prevention of drug-induced histamine release. They help reduce itching in urticaria and atopic dermatitis, but they are not usually effective in asthma. Clinical use of antihistamines is limited by side effects of sedation and mucosal dryness.

H_2 receptor antagonists (eg, cimetidine), used primarily to control gastric acid secretion in peptic ulcer and other conditions of acid hypersecretion, have been reported to relieve chronic urticaria in some patients when used in conjunction with H_1 blocking drugs.

B. Sympathomimetic Amines: The sympathetic nervous system is not primarily involved in the pathogenesis of allergic disease, but in certain allergic reactions—particularly anaphylactic shock and acute asthma—the vascular and visceral effects will evoke a secondary sympathomimetic response to maintain homeostasis of function in the affected organs. Sympathomimetic drugs are therefore highly effective treatment in many manifestations of type I allergy.

Table 24–3. Antihistamines with H_1-receptor blocking effect.

Drug	Usual Adult Dose (4 Times Daily)	Usual Adult Dose (2 Times Daily)
Ethanolamines		
Carbinoxamine	4 mg	...
Clemastine	2.68 mg	...
Diphenhydramine	25 mg	...
Ethylenediamines		
Pyrilamine	25 mg	...
Tripelennamine	15 mg	...
Alkylamines		
Brompheniramine	4 mg	...
Chlorpheniramine	4 mg	...
Dexchlorpheniramine	2 mg	...
Triprolidine	2.5 mg	...
Piperazines		
Hydroxyzine	2.5 mg	...
Phenothiazines		
Promethazine	12.5 mg	...
Trimeprazine	10 mg	...
Others		
Azatadine	1 mg	...
Cyproheptadine	4 mg	...
Terfenadine	...	60 mg

The diverse actions of sympathomimetic amines are explained by 2 classes of receptors—α and β—and their subclasses—α_1, α_2, β_1, and β_2.

α_1-Adrenergic agonists cause mucosal vasoconstriction and are widely used as nasal decongestants. Examples of such drugs are phenylephrine and phenylpropanolamine. For many years, epinephrine and isoproterenol were the principal sympathomimetic bronchodilators available for treatment of asthma, but their use was limited by the cardiac stimulating properties of their α and β_1 agonist effects, respectively. A variety of β_2-selective bronchodilators are now available for treatment of asthma. These include metaproterenol, terbutaline, albuterol, isoetharine, and procaterol. Epinephrine is the drug of choice for anaphylaxis because it has powerful α- and β-stimulating effects necessary to counteract the systemic effects of anaphylaxis.

C. Cromolyn: Cromolyn (disodium cromoglycate) is a unique drug that inhibits allergen-induced release of mediators from human mast cells but not basophils. It interferes through unknown mechanisms with the intracellular events leading to mast cell degranulation. It does not inhibit the peripheral actions of histamine, nor is it a bronchial smooth muscle dilator. It is poorly absorbed and for that reason effective by topical administration only. Cromolyn is available as a dry powder for inhalation in asthma and as topical solutions for nasal and ophthalmic use. Some reports suggest that high-dose oral cromolyn is effective in allergic gastroenteropathy. For unknown reasons, only certain patients respond to cromolyn therapy, but the drug has virtually no side effects or long-term toxicity.

D. Theophylline: The methylxanthines—theophylline, caffeine, and theobromine—stimulate the central nervous system, produce diuresis, stimulate cardiac muscle, and relax bronchial smooth muscle. Theophylline is the most effective of these as a bronchodilator, and it has achieved a prominent though somewhat controversial role in the treatment of asthma. The biochemical explanation for this effect is not currently known.

E. Corticosteroids: Steroid hormones and their synthetic analogs that have glucocorticoid activity exert potent ameliorating effects in almost all types of allergic reactions when administered in supraphysiologic (pharmacologic) doses. In recent years, the pharmacology of these drugs has been traced to a cytoplasmic receptor that binds to the corticosteroid, forming a steroid-receptor complex which enters the nucleus, where it interacts with DNA and directs the synthesis RNA and eventually of specific proteins. The antiallergic effect of steroid therapy, therefore, is

thought to result from inhibition of the activity of inflammatory cells by such an induced protein. The therapeutic effect of steroids in hypersensitivity is not related to inhibition of antibody formation, and the suppression of inflammation by glucocorticoids is not limited to allergic reactions, since nonimmunologic inflammation induced by infection, trauma, or irritants is suppressed as well. A large number of glucocorticoid drugs are available for systemic or local administration. Because of potential irreversible long-term adverse effects, steroid therapy for non-life-threatening diseases should be carefully evaluated and used only after failure of less dangerous forms of treatment.

Immunotherapy

Immunotherapy refers to the repeated injections of allergen in increasing dosage over a prolonged period of time. This treatment is effective for several types of allergic diseases, and it is frequently employed in allergic rhinitis, allergic asthma, and Hymenoptera insect sting anaphylaxis.

Immunotherapy has been shown to reduce symptoms of allergic rhinitis in patients with seasonal pollen allergy and is probably effective also in mold or dust allergy. Several immunologic changes occur during the course of immunotherapy in atopic patients. Circulating IgE antibody increases slightly during the initial months of treatment and then gradually falls below pretreatment levels over a period of several years. However, it is rarely eliminated completely—ie, a true state of desensitization is seldom achieved. Blocking antibody, which is an IgG antibody with specificity for the injected allergen that binds circulating allergen without initiating a type I reaction, appears in the serum of most treated patients. The normal postseasonal rise in IgE antibody to pollens is diminished, suggesting that treatment may induce a form of partial immunologic tolerance. Clinical improvement during immunotherapy correlates better with the blocking antibody response than with other immunologic changes, but a combination of several mechanisms might be required for optimal results.

A. Methods of Treatment: Many injection schedules have been devised, but only 2 are currently in general use: perennial and preseasonal. In the perennial method, the patient continuously receives injections containing a mixture of the relevant allergens. Treatment is begun at a dose low enough to avoid any local or systemic reactions, and frequent injections—usually once or twice a week—at increasing dosages are given until the highest dose the patient can tolerate without excessive local or systemic reactions is reached. This is the maintenance dose, which is then continued at less frequent intervals, usually every 2–6 weeks depending upon the patient's response. If treatment is begun during a pollen season, the starting dose must be quite low to avoid reactions.

In the preseasonal method, frequent injections of increasing doses are administered beginning 3–6 months before the anticipated start of the pollen season, and the treatment is stopped just before the season begins. The same procedure is repeated each year. This method is cumbersome in patients with multiple seasonal allergies.

Because of the large number of injections required in immunotherapy of atopic disease, several attempts have been made to use immunologic adjuvants to reduce the number of injections to as few as one for each season. Freund's incomplete adjuvant, an emulsion of aqueous allergen extract suspended in mineral oil and administered intramuscularly, was given extensive trials in the 1960s but is rarely used today because of concern about possible adverse effects of mineral oil in tissues and lack of evidence for efficacy. Alum-adsorbed allergen extracts are available commercially, but studies have not yet shown that they are superior to aqueous allergens. Chemical modification or polymerization of the allergen molecule to render it less allergenic while retaining or enhancing its immunogenicity for treatment is currently under study. Immunotherapy with polymerized pollen extracts has been shown in clinical trials to produce good blocking antibody levels, achieve control of allergic symptoms, and have little risk of adverse reactions.

B. Injection Technique: The success of immunotherapy using conventional aqueous extracts requires proper technique. Injections are given subcutaneously on the lateral or dorsal aspect of the upper arm. The patient should be observed for 20 minutes afterward, so that a systemic reaction can be treated immediately if it occurs. In the event of a systemic reaction, the next dose should be decreased. Swelling up to 3 or 4 cm in diameter lasting less than 24 hours and accompanied by erythema and itching is to be expected at the maintenance dose level. Local reactions larger than this indicate the need to reduce the dose.

C. Duration of Treatment: The duration of treatment differs for each patient. When injections are continued for several years, most patients report that symptoms lessen with each succeeding year. After 2 successive years with few or no allergic symptoms, it is probably desirable to discontinue the injections, although many patients want to continue on a maintenance dose.

D. Adverse Effects of Immunotherapy: Immunotherapy of atopic disease is effective, and the risk of discomfort or serious reaction is relatively low if treatment is done properly. The principal danger is the immediate systemic reaction, an anaphylactic response to an excessively high dose or inadvertent administration into a blood vessel. The symptoms and treatment of anaphylaxis are discussed below. Reactions to allergy injections can be minimized by scrupulous record-keeping, questioning the patient each time about local reactions from the previous dose, and proper technique of administration. Anaphylactic deaths have occurred from allergy injections, but in some cases this has been attributed to an incorrect dose or improper procedure.

There is no evidence that repeated administration of allergens to atopic patients induces other forms of immunologic disease.

ATOPY

About one in 10 persons in the USA suffers from clinically significant atopic disease. The most common form of the disease is allergic rhinitis, usually seasonal pollen allergy (hay fever). Less frequently, atopic disease is expressed as bronchial asthma or atopic dermatitis, and rarely as gastrointestinal food allergy. The patient may have 2 or more manifestations of the atopic state, but not necessarily at the same time.

Atopic allergy is a type I hypersensitivity reaction to environmental antigens (allergens) in genetically susceptible individuals who produce IgE antibodies to allergens such as pollens, molds, house dust, animal danders, or foods. Exposure to the offending allergen results in the release of mediators, including histamine, leukotrienes (SRS-A), and ECF-A, in the target organ. The action of these mediators on blood vessels, smooth muscle, and secretory glands and the accompanying edema and cellular infiltrate are responsible for the clinical manifestations and pathologic features of the disease. Individual allergic sensitivities can usually be identified accurately, but the clinical manifestations are modified and influenced by many nonimmunologic factors such as infections, emotions, and drugs.

Genetic factors have long been suspected in atopy and are complex. There is a strong familial clustering of cases. IgE antibody responses occur in normal persons, so the phenotypic expression of atopy may be an enhanced absorption or processing of antigen prior to its exposure to IgE antibody-forming cells. There is evidence that specificities of IgE antibodies in hay fever are determined by immune response genes closely linked to the HLA histocompatibility gene complex. Total serum IgE concentration, which is typically elevated in atopy, is controlled by a separate gene not linked to the HLA complex.

ALLERGIC RHINITIS (Hay Fever)

Major Immunologic Features

- Allergic rhinitis is the most common clinical expression of atopic hypersensitivity.
- Type I allergy localized in the nasal mucosa and conjunctiva.
- Pollens, fungal spores, dust, and animal danders are the usual atmospheric allergens.

General Considerations

Allergic rhinitis is the most common manifestation of an atopic reaction to inhaled allergens. At least 20 million persons in the USA suffer from this disease. Onset may be at any age but is usually during childhood or adolescence.

The immunologic pathogenesis of allergic rhinitis is discussed above in the section on atopy.

Clinical Features

A. Symptoms and Signs: A typical attack consists of symptoms of profuse watery rhinorrhea, paroxysmal sneezing, and nasal obstruction. Itching of the nose and palate is common. There is frequently an accompanying allergic blepharoconjunctivitis, with intense itching of the conjunctiva and eyelids. In some patients, conjunctivitis may occur in the absence of nasal symptoms. The disease occurs seasonally in patients with pollen allergy; may be present year-round if the sensitivity is to a perennial allergen such as house dust; or there may be perennial symptoms with seasonal exacerbations in patients with multiple allergies. Severe attacks are often accompanied by systemic symptoms of malaise and sometimes muscle soreness after intense periods of sneezing. Fever is absent. Swelling of the nasal mucosa may lead to headache because of obstruction of the ostia of the paranasal sinuses.

Examination shows a pale, swollen nasal mucosa with watery secretions. The conjunctiva is suffused or injected, and the lids are frequently swollen. These changes revert to normal when there is no allergen exposure and the patient is asymptomatic.

B. Laboratory Findings: Eosinophils are numerous in the nasal secretions, and blood eosinophilia is present during symptomatic periods. Serum IgE is modestly elevated but may be normal.

Immunologic Diagnosis

The diagnosis of allergic rhinitis is based on the history, physical findings during the symptomatic phase, and nasal eosinophilia. Wheal and erythema skin tests will detect the specific sensitivities. (See section above on skin testing.)

Differential Diagnosis

Chronic vasomotor rhinitis is a common disorder of unknown cause in which the primary complaint is nasal congestion, usually associated with postnasal drainage. It differs from allergic rhinitis in that there are no sneezing paroxysms or eye symptoms and minimal rhinorrhea. Congestion may be unilateral or bilateral and often shifts with position. Symptoms occur year-round and are generally worse in cold weather or in dry climates. The nasal mucosa is unusually sensitive to irritants such as tobacco smoke, fumes, and smog. Symptoms usually begin in adult life, and the disease is more common among women. It may begin during pregnancy. Examination shows swollen, erythematous nasal mucosa and strands of thick mucoid postnasal discharge in the pharynx. Allergy skin tests are negative or unrelated to the symptoms. In nonallergic vasomotor rhinitis, the nasal secretions may or may not contain eosinophils, so nasal eosinophilia is not a reliable sign of allergy but may indicate a preasthmatic state. There is a good response to decongestants and humidification, but antihistamines are usually not effective.

Rhinitis medicamentosa denotes the severe congestion that occurs from the rebound effect of sympath-

omimetic nasal sprays or nose drops used excessively. In this disease, the mucosa is often bright red and swollen, but these changes are reversible with complete avoidance of nose drops or sprays even if they have been used excessively for many years.

Infectious rhinitis is almost always due to a virus, and most patients with allergic rhinitis can distinguish their allergic symptoms from those of the common cold, which usually produces fever, an erythematous nasal mucosa, and a polymorphonuclear rather than eosinophilic exudate in the nasal secretions. Primary bacterial or fungal infections of the nasal passages are rare.

Treatment

Treatment consists of environmental measures to avoid allergen exposure, drugs, and immunotherapy. Environmental therapy is discussed above.

A. Drug Treatment: Antihistaminics are the most useful drugs in allergic rhinitis, although their use is restricted by side effects, especially sedation. Nasal decongestants may be helpful, either alone or in combination with antihistaminics. Sympathomimetic eye drops are useful for allergic conjunctivitis. Cromolyn by nasal sprays or conjunctival drops is beneficial for some patients.

Corticosteroids can be extremely effective in relieving symptoms of allergic rhinitis; but since the disease is a chronic, recurrent, benign condition, these drugs should be used with extreme care. The patient with very severe symptoms lasting for only a few days or several weeks each year who does not respond to antihistaminics can be given oral prednisone for 1 or 2 weeks in a dosage just high enough to supress symptoms for that patient. Flunisolide or beclomethasone by nasal spray may be equally effective, and they do not cause a significant systemic corticosteroid effect. These drugs can probably be used on a long-term basis, because adverse effects on the nasal mucosa have not been reported.

B. Immunotherapy: Immunotherapy has been shown to be effective in allergic rhinitis. Because of the length of treatment required and the potential danger of serious systemic reactions, injection treatment is used in patients whose symptoms are uncontrolled in spite of appropriate environmental measures and symptomatic medications. This is discussed in detail above.

Complications & Prognosis

Purulent sinusitis and otitis media can result from obstruction of the sinus ostia or auditory (eustachian) tubes, respectively. The development of nasal polyps is not directly related to the severity of the allergic disease.

Although no definitive studies have been done on the course of untreated allergic rhinitis, symptoms can be expected to recur or persist for many years if not for life, although the severity of the symptoms is dependent upon the degree of exposure to the allergen. A patient with pollen allergy who moves to an area where the offending plant does not grow will no longer be symptomatic.

ASTHMA

Major Immunologic Features

- Allergic asthma is a manifestation of type I allergy localized in the bronchus.
- Immunologically released or activated mediators are histamine, leukotrienes, and ECF-A.
- Hyperirritability of bronchial mucosa amplifies the bronchoconstricting effects of mediators.

General Considerations

Bronchial asthma is a chronic disease characterized by hyperirritability of the bronchial mucosa and eosinophilia. It may begin at any age and results in attacks of wheezing and dyspnea that can range in severity from mild discomfort to life-threatening respiratory failure. Some patients are symptom-free between attacks, whereas others are never entirely free of airway obstruction.

A. Extrinsic Asthma (Allergic, Atopic, or Immunologic Asthma): About 50% of asthmatics have evidence of atopic allergy. As a group, they generally develop the disease early in life, usually in infancy or childhood. Other manifestations of atopy—eczema or allergic rhinitis—often coexist. A family history of atopic disease is common. Attacks of asthma occur during pollen seasons, in the presence of animals, or on exposure to house dust, feather pillows, or other allergens, depending upon the patient's particular allergic sensitivities. Skin tests give positive wheal-and-flare reactions to the causative allergens. Total serum IgE concentration is frequently elevated but is sometimes normal.

B. Intrinsic Asthma (Nonallergic or Idiopathic Asthma): This type of asthma characteristically appears first during adult life, usually after an apparent respiratory infection, so that the term "adult-onset asthma" is sometimes applied to this disorder. This term is misleading because in some cases the disease first appears during childhood and because some allergic asthmatics who have not previously been exposed to the relevant allergen become symptomatic for the first time as adults. Intrinsic asthma pursues a course of chronic or recurrent bronchial obstruction unrelated to pollen seasons or exposure to other allergens. Skin tests are negative to the usual atopic allergens. Serum IgE concentration is normal. Personal and family histories are usually negative for other atopic diseases.

Approximately 10% of asthmatic patients have aspirin sensitivity. In these patients, ingestion of aspirin is followed in 20 minutes to 3 hours by an asthmatic attack, which is caused by an idiosyncratic pharmacologic response to the drug. In some cases, other nonsteroidal anti-inflammatory drugs cause a similar reaction. Nasal polyposis is common in aspirin-sensitive patients.

Immunologic Pathogenesis

The cause of asthma is not known. There is evidence that bronchoconstriction is mediated by an autonomic reflex mechanism involving afferent receptors in the bronchial mucosa or submucosa that respond to irritants or chemical mediators and efferent cholinergic (vagal) impulses causing bronchial muscle contraction and hypersecretion of mucus. In the asthmatic patient, the afferent receptors appear to be sensitized to respond to a low threshold of stimulation. It has been proposed that the hyperirritable state of the bronchial mucosa results from defective functioning or blockade of its β-adrenergic receptor, preventing a homeostatic bronchodilating response from endogenous catecholamines.

The abnormality is presumably the same in all asthmatics, differing only in degree. In allergic asthma, allergen-induced attacks can be initiated by direct reaction between inhaled allergen and IgE antibody on bronchial mast cells, releasing histamine and leukotrienes to stimulate local bronchial receptors, or indirectly by allergen or mediators reaching the site via the circulation if the allergen is ingested or injected.

The mechanism of aspirin-sensitive asthma is idiosyncratic and not immunologic. Since aspirin and related compounds normally inhibit the cyclooxygenase pathway of biosynthesis of prostaglandin E_2 (a bronchodilator) from arachidonic acid, it is suspected that in this disease an aberrant response to these drugs favors the local synthesis of prostaglandin E_2 (a bronchoconstrictor) or leukotrienes via the lipoxygenase pathway.

Clinical Features

A. Symptoms and Signs: The asthmatic attack causes shortness of breath, wheezing, and tightness in the chest, with difficulty in moving air during both inspiration and expiration. Coughing is usually present, and with prolonged asthma the cough may produce thick, tenacious sputum that can be either clear or yellow. Physical examination during the attack shows tachypnea, audible wheezing, and use of the accessory muscles of respiration. The pulse is usually rapid, and blood pressure may be elevated. Pulsus paradoxus indicates severe asthma. The lung fields are hyperresonant, and auscultation reveals diminished breath sounds, wheezes, and rhonchi but no rales.

B. Laboratory Findings: Blood and sputum eosinophilia are characteristic of asthma, whether or not allergy is present. The chest x-ray may be normal during the attack or may show signs of hyperinflation, and there may be transient scattered parenchymal densities indicating focal atelectasis caused by mucous plugging in scattered portions of the airway.

Pulmonary function tests show the abnormalities of airway obstructive disease. Flow rates and FEV_1 are decreased, vital capacity is normal or decreased, and total lung capacity and functional residual capacity are increased over normal values. Diffusing capacity is usually normal or slightly increased but may be decreased with extreme bronchospasm. Following administration of an aerosolized sympathomimetic bronchodilator, ventilation improves, with significant increase in flow rates and FEV_1, indicating the reversible nature of the bronchial obstruction. The lack of response in a patient already receiving large doses of sympathomimetic drugs does not rule out reversibility, and the test should be repeated at a later date after additional treatment such as hydration, corticosteroids, and chest physical therapy

Repeated tests of ventilatory function are helpful in the long-term management of the asthmatic patient. Serial determinations of FEV_1, maximal expiratory flow rate (MEFR), or peak flow rates are easily done in the office or clinic, and they will often detect airway obstruction that may not be apparent to the patient or to the physician on auscultation of the chest.

Increased total eosinophil count in the peripheral blood is almost invariably present unless suppressed by corticosteroids or sympathomimetic drugs. Sputum examination reveals eosinophils, Charcot-Leyden crystals, and Curschmann's spirals.

Immunologic Diagnosis

The diagnosis of bronchial asthma is based on the history, physical examination, and pulmonary function tests. The history is the primary diagnostic tool for evaluating the presence of allergy and identifying the relevant allergens. In general, those inhalant allergens that are important in allergic rhinitis are also implicated in allergic asthma: pollens, fungi, animal danders, house dust, and other household and occupational airborne allergens. In young children and infants, allergy to foods may also cause asthma. If atopic allergy is suggested by the history, skin testing for wheal-and-flare reactions will verify the specific sensitivities. Bronchoprovocation allergen testing is used primarily in difficult diagnostic cases of suspected occupational lung disease.

Differential Diagnosis

Chronic bronchitis and emphysema (chronic obstructive lung disease) produce airway obstruction that does not respond to sympathomimetic bronchodilators or corticosteroids, and there is no associated eosinophilia in the blood or sputum. In children, acute bronchiolitis, cystic fibrosis, aspiration of a foreign body, and airway obstruction caused by a congenital vascular anomaly must be considered. Benign or malignant bronchial tumors or external compression from an enlarged substernal thyroid, thymus enlargement, aneurysm, or mediastinal tumor may cause wheezing. Acute viral bronchitis may produce enough bronchial inflammation with symptoms of obstruction and wheezing to be called asthmatic bronchitis. Cardiac asthma is a term used for intermittent dyspnea (resembling allergic asthma) caused by left ventricular failure. Carcinoid tumors may occasionally cause attacks of wheezing because of release of serotonin or activation of kinins by the neoplasm.

Treatment

Since the cause of asthma is unknown, cure of the basic defect, the hyperirritable bronchial mucosa, is not possible. The aim of treatment is symptomatic control. Drugs, environmental measures, and immunotherapy may be required.

A. Drug Treatment:

1. Sympathomimetics—Adrenergic bronchodilator drugs are effective treatment and are used in the acute attack or for long-term management. Epinephrine, 0.2–0.5 mL of 1:1000 aqueous solution given subcutaneously, acts rapidly and should be the first drug used for the acute attack. Its duration of action is short, so that if repeated injections are required, long-acting epinephrine 1:200 (epinephrine suspension), epinephrine in oil (2 mg/mL), or terbutaline can be used. Epinephrine can also be given by inhalation as an aerosol, but albuterol, metaproterenol, or isoetharine is preferable because these drugs have a predominantly β-adrenergic activity. They are available as solutions to be administered by a hand-held nebulizer or in an intermittent positive-pressure breathing (IPPB) device. They are also dispensed in convenient metered-dose pressurized inhalers, but patients must be cautioned that overuse can lead to paradoxic bronchial constriction and worsening of the asthma. Deaths from isoproterenol aerosol abuse have been reported.

β-Adrenergic drugs—terbutaline, metaproterenol, and albuterol—have largely supplanted ephedrine as oral sympathomimetic drugs for achieving sustained bronchodilatation in chronic asthma. Side effects of nervousness, muscle twitching, palpitations, tachycardia, and insomnia can occur with all of these.

2. Xanthines—Theophylline and related compounds are bronchodilators especially effective when used in combination with sympathomimetic drugs. Intravenous aminophylline, 250–500 mg, can be administered fairly rapidly in the acute asthmatic attack, and various oral forms of theophylline are available for long-term use. Absorption of theophylline varies with the drug preparation, the age of the patient, and other factors such as smoking and heart failure. Serum theophylline determinations should be utilized to obtain a therapeutic level of 10–20 μg/mL.

3. Corticosteroids—Glucocorticoids are remarkably effective in the treatment of asthma. Even when all other forms of treatment have failed, the response to adequate steroid treatment is so dependable that failure of response might be considered grounds for questioning the diagnosis of asthma. The mechanism of action is unknown, and these drugs are just as effective in reversing asthma in nonallergic patients as in patients suffering allergen-induced attacks.

In spite of their effectiveness, however, corticosteroids should not be considered primary agents in the treatment of asthma, and in actual practice they should be given only when other forms of treatment prove inadequate. The dangers of long-term steroid therapy must be kept in mind by any physician prescribing the drugs.

Treatment is started at high dosage and continued until the obstruction is alleviated, with return of physical findings and flow rates to normal. The dose necessary to achieve this varies with the individual patient, but 30–60 mg of prednisone daily is usually sufficient. An occasional steroid-resistant patient may require a much higher dose because of an abnormally accelerated rate of drug catabolism. After complete clearing of the attack, the daily dose is reduced by slow tapering over many days or weeks to avoid a flare-up. Long-term maintenance therapy is required by some patients, using a dose as low as possible to maintain symptomatic control, usually at a level of 10–15 mg of prednisone daily. Single-dose alternate-day maintenance therapy minimizes adrenocortical suppression, but not all steroid-dependent asthmatic patients can be controlled in this fashion.

Beclomethasone dipropionate, a highly potent corticosteroid drug available in aerosolized form for inhalation, is effective for long-term maintenance therapy for many steroid-dependent asthmatic patients. When beclomethasone is used in a daily dosage of less than 1000 μg, adrenocortical suppression and systemic side effects are virtually absent. The starting dose is 400 μg daily, with subsequent dosage adjustment to meet the patient's requirement, but the previously used systemic steroid drug must be tapered very slowly to avoid adrenal insufficiency. Inhaled beclomethasone is not useful for treatment of an acute attack.

4. Cromolyn sodium—This drug is available as a powder administered in 20-mg doses by inhalation using a specially designed inhaler. It is not a bronchodilator but is believed to inhibit release of mediators of immediate hypersensitivity in the lung. It is administered as long-term prophylactic treatment. It is more effective in younger patients with allergic asthma than in adults, and it frequently prevents exercise-induced bronchospasm. Cromolyn will not reverse the acute attack.

5. Other drugs—Antibiotics are used if secondary bacterial bronchitis or pneumonia occurs. Expectorants and hydration are helpful for thick, tenacious sputum.

B. Environmental Control: Irritants such as smoke, fumes, dust, and aerosols should be avoided. If the diagnostic evaluation indicates allergy to animal danders, feathers, molds, or house dust, these should be eliminated from the house.

C. Immunotherapy: The effectiveness of injection treatment in pollen hay fever has been shown in several controlled studies, and most allergists feel that allergic asthma responds just as well. (See section on immunotherapy, above.)

D. Treatment of Status Asthmaticus and Respiratory Failure: A severe attack of asthma unresponsive to repeated injections of epinephrine or other sympathomimetic drugs, termed status asthmaticus, is a medical emergency requiring immediate hospitalization and prompt treatment. Factors leading to this condition include respiratory infection, excessive

use of respiratory depressant drugs such as sedatives or opiates, overuse of aerosolized bronchodilators, rapid withdrawal of corticosteroids, and ingestion of aspirin in an aspirin-sensitive asthmatic patient.

Immediate determination of arterial blood gases and pH with repeated measurements until the patient responds satisfactorily is necessary for optimal treatment. Injections of epinephrine, epinephrine suspension, or epinephrine in oil are continued. Aminophylline, 250–500 mg, is given intravenously over a period of 10–30 minutes initially, followed by slow intravenous drip, but careful attention must be paid to toxic symptoms of nausea, vomiting, or headache. Serum theophylline determinations are useful in maintaining the optimal therapeutic level of 10–20 μg/mL of serum. Intravenous corticosteroids are indicated if the patient has previously received steroids, if the attack was caused by aspirin, if excessive aerosolized isoproterenol was a factor in the attack, or if significant CO_2 retention exists. Intravenous hydrocortisone, 4 mg/kg, or methylprednisolone, 1 mg/kg, repeated every 2–4 hours, should be given until the patient can be maintained on oral prednisone, 60–80 mg daily in divided doses.

Dehydration usually accompanies status asthmaticus and may give rise to inspissated mucus plugs that further impair ventilation. During the first 24 hours, up to 3–4 L of intravenous fluid may be necessary for rehydration. Oxygen should be supplied by tent, face mask, or nasal catheter to maintain arterial P at about 90–100 mm Hg. Expectorants and chest physical therapy are helpful adjuncts to eliminate mucus plugs. Sedatives should be avoided even in the anxious patient because of the danger of respiratory depression. Antibiotics are used only for concomitant bacterial infection.

Respiratory failure, indicated by an arterial P level of 65 mm Hg or more and arterial blood pH below 7.25, may require mechanical assistance of ventilation in addition to all the measures listed above. This should be performed by a team of physicians, nurses, and technicians experienced in respiration therapy.

Complications & Prognosis

The disease is chronic, and its severity may change in an unpredictable fashion. Some children apparently "outgrow" asthma in the sense of becoming asymptomatic, but they will continue to show evidence of bronchial lability, and symptoms can reappear later in life. The acute attack can be complicated by pneumothorax, subcutaneous emphysema, rib fractures, atelectasis, or pneumonitis. There is no evidence that emphysema, bronchiectasis, pulmonary hypertension, or cor pulmonale results from long-standing uncomplicated asthma.

Allergic bronchopulmonary aspergillosis: This disease occurs almost exclusively in patients with a history of asthma who harbor *Aspergillus* endobronchially and who develop a heterogeneous form of hypersensitivity with both IgE and IgG antibodies to *Aspergillus* antigens. (See Chapter 26.)

ATOPIC DERMATITIS

Major Immunologic Features

- Often accompanies atopic respiratory allergy.
- Clinical course usually independent of allergen exposure.
- Very high serum level of IgE may occur.

General Considerations

Atopic dermatitis is associated with allergic rhinitis and asthma in families and frequently in the same patient, suggesting that it is a cutaneous form of atopic hypersensitivity. Furthermore, serum IgE is usually very high. However, it is often difficult to prove that allergy plays a role, because the severity of the dermatitis does not usually correlate with exposure to allergens to which the patient reacts positively on skin testing, and immunotherapy is not effective in this disease. There is evidence for an underlying target organ (skin) abnormality that might be a metabolic or biochemical defect, possibly linked genetically to the high level of serum IgE. Some studies also suggest a partial deficiency in T cell immunity.

Atopic dermatitis may begin at any age. Onset in infancy at 3–6 months is typical, but it may first appear during childhood or adolescence and occasionally during adult life.

Immunologic Pathogenesis

A. The Role of Allergy in Atopic Dermatitis: Atopic respiratory diseases with hypersensitivity to environmental allergens, eosinophilia, elevated serum IgE levels, and a family history of allergy are frequently associated with atopic dermatitis. Nevertheless, it is often difficult to attribute the dermatitis to allergy. The skin lesions rarely flare during pollen seasons, although in some patients there is an association with exposure to house dust, animals, or other environmental allergens. More commonly, food allergy in children can be demonstrated. Milk, corn, soybeans, fish, nuts, and cereal grains are frequently implicated, but other foods may occasionally be important allergens also.

B. Association With Systemic Disorders: Eczema indistinguishable from atopic dermatitis is found in children with phenylketonuria. The skin lesions of Letterer-Siwe disease are also very similar. Atopic dermatitis without allergy is a feature of several immunologic deficiency disorders, especially Wiskott-Aldrich syndrome, ataxia-telangiectasia, and X-linked hypogammaglobulinemia.

Clinical Features

Dry skin and pruritus are the essential abnormalities in the skin. They lead to chronic scratching and rubbing, producing the characteristic features of eczema. In infancy, the forehead, cheeks, and extensor surfaces of the extremities are usually involved, but later the lesions show a flexural pattern of distribution, with predilection for the antecubital and popliteal areas and the neck. The face, especially around the

eyes and ears, is often affected when distribution is more widespread. The skin is excessively dry. Active lesions are initially erythematous and pruritic. This leads to scratching, which results in excoriations, papules, and scaling. If treated promptly, these changes revert to normal; but with prolonged scratching the skin becomes lichenified and pigmentation is altered. The disease often improves spontaneously during the summer months.

Differential Diagnosis

Generalized neurodermatitis, localized neurodermatitis (lichen simplex chronicus), and contact dermatitis produce similar eczematous changes of the skin. Seborrhea and dermatophytoses are occasionally confused with atopic dermatitis.

Treatment

Atopic dermatitis is a chronic disease requiring constant attention to proper skin care, environmental control, drugs, and avoidance of allergens when indicated. Because dry skin enhances the tendency to itch, frequent application of nonirritating topical lubricants is the most important preventive measure. Areas involved with active eczema respond well to topical corticosteroids, but acute involvement of large areas of skin may warrant a rief course of systemic corticosteroids beginning with a high dose initially and tapering slowly after the acute eruption clears. Oral antihistaminics help to control itching. Even if their sedative effect precludes use during the daytime, a bedtime dose will help to control involuntary scratching during sleep. Frequent bathing or washing, irritating fabrics such as wool, and harsh detergents should be avoided. The hands and fingernails must be kept clean to prevent secondary infection, and if infection does occur an appropriate antibiotic should be prescribed.

Complications & Prognosis

Atopic dermatitis has an unpredictable tendency to remit spontaneously, even after years of involvement, and this is not related to the severity of involvement, the presence or absence of allergy, or treatment. Allergic rhinitis and asthma are not complications but rather additional manifestations of the underlying atopic disease.

The most frequent complication is secondary infection from scratching. In the past, the most serious complication was eczema vaccinatum from exposure to vaccinia virus by inadvertent vaccination or contact with a recently vaccinated person in the family or classroom. Eczema herpeticum is a similar condition caused by herpes simplex virus. Topical antibiotics or antihistamines may cause secondary contact dermatitis. Cataracts occur in a small number of cases; the cause is unknown.

ALLERGIC GASTROENTEROPATHY

Major Immunologic Features

- Some atopic patients have localized IgE reactions in the gut to an ingested food.
- Gastrointestinal loss of serum proteins and blood may lead to edema and anemia.
- Rare in adults; more common but transient in infants.

General Considerations

Allergic gastroenteropathy is the least common expression of atopy. Ingested food allergen reacting with local IgE antibodies in the jejunal mucosa liberates mast cell mediators which produce gastrointestinal symptoms shortly after the meal. Continued exposure to the food produces chronic inflammation, resulting in gastrointestinal protein loss and hypoproteinemic edema. Blood loss through the inflamed intestinal mucosa may be significant enough to cause iron deficiency anemia. In some patients, extra-enteric manifestations of atopy may be produced by the same food allergen.

Immunologic Pathogenesis

The pathogenesis is that of atopy, as discussed above. The condition may occur more commonly in infants than in adults because of the much greater permeability of infantile gastrointestinal mucosa to intact proteins. This may account for the transient nature of allergic gastroenteropathy in infants and young children.

Clinical Features

Diarrhea, vomiting, and abdominal pain occur, usually less than 2 hours after ingestion of the allergenic food. Chronic exposure to the food when the disease is unrecognized may lead to growth retardation in children. The allergen-induced enhanced gastrointestinal permeability causes a loss of serum proteins, leading to generalized edema in some patients. Gastrointestinal blood loss may also occur, manifested by hypochromic microcytic iron deficiency anemia. Eosinophilia in blood and gastrointestinal secretions accompanies the allergic reaction when the food allergen is eaten. Charcot-Leyden crystals may appear in the stool. Gastrointestinal x-rays are normal or show small bowel edema.

Most patients have other manifestations of atopy, including atopic dermatitis, asthma, and allergic rhinitis, and there is usually a family history of allergy. Milk is the usual allergen in the childhood form of the disease, but adults may react to other foods. In each of the few reported cases, the causative food allergens are limited to one or a small number of foods. Nursing infants may react to food allergens in breast milk from the maternal diet.

Immunologic Diagnosis

A history of gastrointestinal symptoms occurring 30–120 minutes after eating is suggestive of the disease in a patient with other manifestations of atopy and a high total serum IgE. The suspected food allergen can be identified by history and confirmed by elimination and challenge with the suspected food, preferably

performed double-blind. Jejunal biopsy will show eosinophils in the lamina propria during the reaction. The allergenic food will usually produce a type I immediate wheal-and-erythema skin test.

Differential Diagnosis

Gastrointestinal allergy is overdiagnosed. Patients with food-related gastrointestinal symptoms—even atopic patients—are much more likely to have nonallergic food intolerance. Primary gastrointestinal diseases, reactions to food contaminants, and psychologic food aversions must be considered. Inflammatory bowel diseases, intestinal lymphangiectasia, and primary immunoglobulin deficiencies may produce similar symptoms. In children, lactase and other carbohydrate enzyme deficiencies, phenylketonuria, pancreatic deficiency from cystic fibrosis, and maple syrup urine disease should be ruled out by appropriate tests.

Treatment

Elimination of the allergenic food from the diet is curative. In some cases of milk allergy, boiled milk may be tolerated if the protein allergen is heat-labile. Corticosteroid treatment usually inhibits the reaction, but long-term steroid therapy to permit a patient to eat an allergenic food is almost never indicated. There are reports that oral cromolyn in a dose of 200–400 mg prior to ingesting the allergenic food inhibits the gastrointestinal allergic reaction.

Complications

The major complications of this disease are edema and anemia. Unlike intestinal lymphangiectasia, gastroenteric loss of plasma immunoglobulins and lymphocytes does not occur, so susceptibility to infection is usually not a problem. Persistent disease activity may lead to secondary reversible lactose intolerance.

Prognosis

The infantile form of allergic gastroenteropathy is usually transient, but the duration of disease is unpredictable and not related to the severity of the reaction. No long-term follow-up studies on adults are available.

ANAPHYLAXIS

Major Immunologic Features

- Systemic anaphylaxis is the occurrence of a type I reaction simultaneously in multiple organs.
- The usual causative allergen is a drug, insect venom, or food.
- The reaction can be evoked by a minute quantity of allergen and is potentially fatal.

General Considerations

Anaphylaxis is a systemic form of immediate hypersensitivity affecting several organ systems simultaneously. The reaction occurs rapidly and may cause death through respiratory obstruction or irreversible vascular collapse. Systemic anaphylaxis is usually mediated by IgE antibodies, with release of histamine and leukotrienes, but IgG- or IgM-mediated complement-dependent mechanisms generating anaphylatoxins or kinins may account for some anaphylactic reactions.

In most cases, anaphylaxis is a systemic effect of type I allergy. The allergen combines with IgE antibodies on mast cells, releasing histamine from its stores within mast cell granules and generating leukotrienes from arachidonic acid in membrane phospholipid. The vasodilating, permeability-increasing, and smooth muscle-constricting properties of these chemical mediators account for most of the pathophysiologic changes in anaphylaxis.

Histamine release may also occur in the absence of IgE antibody, but the clinical significance is uncertain IgG or IgM antibodies that activate the complement system can generate anaphylatoxins C3a and C5a, which are cleavage products of C3 and C5 capable of stimulating mast cell release of histamine. In cases of profound vascular collapse, it is likely that kinins—oligopeptides with vasodilating activity—are activated from plasma kininogen.

The allergen-IgE antibody-mast cell-mediator pathogenesis of anaphylaxis is the same mechanism responsible for atopy, but the allergens and route of exposure differ, and genetic factors and target organ hyperresponsiveness are not present in anaphylaxis.

The most common sources of allergens causing anaphylaxis are drugs, foods, and insect stings (Table 24–4).

Anaphylactic sensitivity to Hymenoptera venom

Table 24–4. Common causes of anaphylaxis.

Drugs
Proteins (presumably complete antigens)
Foreign serum
Vaccines
Allergen extracts
Enzymes
Nonprotein drugs (presumably haptens)
Penicillin and other antibiotics
Sulfonamides
Local anesthetics
Salicylates
Foods
Legumes (especially peanuts)
Nuts
Berries
Seafoods
Egg albumin
Stinging insects
Honeybees
Wasps
Hornets
Yellow jackets
Fire ants

can be easily identified, and patients can be protected from future potentially fatal reactions by desensitization and simple protective measures.

The sting of a single insect is sufficient to produce a severe, even fatal anaphylactic reaction in sensitive patients. Sensitization occurs from prior stings, and if patients are allergic to a common or cross-reacting antigen they may have an anaphylactic reaction should they be stung by any species of Hymenoptera insect. There is no evidence that other allergic disease, including atopy and drug anaphylaxis, predisposes to Hymenoptera anaphylaxis.

Clinical Features

The reaction begins within seconds or minutes after exposure to the allergens. There may be an initial fright or sense of impending doom, followed rapidly by symptoms in one or more target organ systems: cardiovascular, respiratory, cutaneous, and gastrointestinal.

The cardiovascular response may be peripheral or central. Hypotension and shock are symptoms of generalized arteriolar vasodilatation and increased vascular permeability producing decreased peripheral resistance and leakage of plasma from the circulation to extravascular tissues, thereby lowering blood volume. In some patients without previous heart disease, cardiac arrhythmias may occur. Death can result from blood volume depletion and irreversible shock or from a cardiac arrhythmia.

The respiratory tract from the nasal mucosa to the bronchioles may be involved. Nasal congestion from swelling and hyperemia of the nasal mucosa and profuse watery rhinorrhea with itching of the nose and palate simulate an acute hay fever reaction. The hypopharynx and larynx are especially susceptible, and obstruction of this critical portion of the airway by edema is responsible for some of the respiratory deaths. Bronchial obstruction from bronchospasm, mucosal edema, and hypersecretion of mucus results in an asthmalike paroxysm of wheezing dyspnea. Obstruction of the smaller airways by mucus may lead to respiratory failure.

The skin is a frequent target organ, with generalized pruritus, erythema, urticaria, and angioedema. Occasionally, urticaria may persist for many weeks or months after all other symptoms have subsided.

Gastrointestinal involvement occurs because of contraction of intestinal smooth muscle, resulting in crampy abdominal pain and sometimes nausea or diarrhea. Similarly, uterine muscle contraction may cause pelvic pain.

Anaphylactoid reactions: The clinical manifestations of anaphylaxis can occur in the absence of any evidence of an allergen-IgE antibody event. These are called "anaphylactoid" reactions and are believed to arise through the nonimmunologic release of vasoactive and inflammatory mediators in certain susceptible individuals. Intravenous radiographic contrast media, aspirin, chymopapain, and other drugs and diagnostic agents cause such reactions. Exercise-induced anaphylaxis has been described recently, and other cases are idiopathic.

Immunologic Diagnosis

A history of symptoms and signs of anaphylaxis immediately after an insect sting, after parenteral administration of a drug or vaccine, or following the ingestion of a drug or food likely to cause anaphylaxis is sufficient to make the diagnosis.

Occasionally, a reaction occurs after injection of 2 agents with high anaphylactic potential (eg, penicillin and horse serum) or after a meal including several different "allergenic" foods such as fish, legumes, nuts, or berries. A positive immediate skin test to the suspected drug or food is probably diagnostic, but a negative test to these allergens never excludes sensitivity. Prick testing should always be done first, followed by serial-dilution intradermal tests if the prick test is negative. If the cause of the reaction is obvious from the history, skin testing should not be done.

The diagnosis of Hymenoptera venom anaphylaxis is made by history and skin testing. Anaphylaxis begins immediately after an insect sting, although mild reactions may not begin until several hours after the sting. Precise insect identification is possible only if the insect is caught and saved for study by an expert. The diagnosis of Hymenoptera insect anaphylaxis is confirmed by positive wheal-and-erythema intradermal test to the specific venom at a concentration of less than 1 μg/mL. RAST is less sensitive but may be employed prior to skin testing if exquisite sensitivity is suspected. However, immunotherapy should not be undertaken on the basis of RAST results alone.

IgE antibodies to major and minor penicillin allergy determinants are detected by wheal-and-flare skin tests. Penicilloyl-polylysine, 6×10^{-5} mol/L solution, elicits a positive skin test in most patients with a history of late urticaria and many of those with exanthematous reactions. A "minor determinant mixture" for testing suspected anaphylactically sensitive patients is not presently marketed, but a skin test using penicillin G, 1000 units/mL, is usually positive in persons with documented anaphylaxis. The tests are performed by injecting 0.005 mL intradermally and reading for wheal and erythema at 20 minutes.

Skin testing with these 2 reagents has predictive value if used in relation to the patient's history. If there is a history of penicillin anaphylaxis, the diagnosis is certain, and no tests should be done, since anaphylaxis can be produced by the test. If late urticaria or skin eruptions are suspected on the basis of the history, negative reactions to both determinants strongly suggest that the patient is not allergic to penicillin and that the drug can be administered with no greater risk of reaction than in those patients who had not received penicillin before. If the minor determinant test is positive, the risk of anaphylaxis is very high, and if the major determinant test is positive the chance of a skin eruption is very high. However, the appearance of late-onset urticaria does not always signify that the drug must be withdrawn or withheld, since continued

treatment may result in the appearance of IgG blocking antibody, causing the urticaria to disappear without interfering with the therapeutic effect of the drug. Hemagglutination tests have been used to detect serum IgG or IgM antibody. The former can function as a blocking antibody, but either might produce type II hemolytic anemia if the drug is given in high dosage intravenously.

Differential Diagnosis

Cardiogenic, hypovolemic, septic, and neurogenic causes of shock must be considered. Acute respiratory obstruction may occur in asthma, pulmonary edema, mechanical obstruction by foreign body or tumor, or adult respiratory distress syndrome. Some patients experience vasovagal syncope after injections, particularly of local anesthetics.

Treatment

Speed is essential, but treatment must be individualized according to organ involvement. Epinephrine should always be given as soon as anaphylaxis is suspected and the patient then examined carefully to determine what further measures are needed. The severity of the reaction is inversely related to the interval between exposure to the allergen and the onset of symptoms.

A. Immediate Treatment: Give epinephrine, 1:1000 aqueous solution, 0.2–0.5 mL intramuscularly into the deltoid muscles. Repeat every 30–60 minutes as necessary, or use a long-acting preparation, such as epinephrine suspension (1:200) or epinephrine in oil, 2 mg/mL. If the reaction was caused by an injected drug or insect sting, give 1:1000 aqueous epinephrine, 0.1–0.2 mL subcutaneously at the injection site, to slow down absorption. If the injection was into an extremity, apply a tourniquet proximally.

B. Hypotension and Shock: Restoration of circulating fluid volume is definitive, but initial vasopressors such as levarterenol bitartrate or metaraminol bitartrate given intravenously may be used. Monitor blood pressure. Give 1000–2000 mL physiologic saline or 5% glucose in saline rapidly intravenously. If there is no response, use plasma or other plasma expanders. If the patient is in profound shock, give whole blood while monitoring central venous pressure.

C. Laryngeal Edema: Maintain the airway. Passage of an endotracheal tube may be difficult because of the swelling, in which case tracheostomy should be performed. Continue epinephrine and give diphenhydramine, 50–100 mg intravenously.

D. Bronchospasm: Treat as for status asthmaticus. Aminophylline, 250–500 mg intravenously, should be administered over a 10-minute period while observing carefully for signs of gastrointestinal or central nervous system toxicity. An aerosolized beta-adrenergic bronchodilator can be administered by hand nebulizer or intermittent positive pressure breathing device.

E. Urticaria, Angioedema, and Gastrointestinal, Genitourinary, or Uterine Symptoms: These respond well to antihistaminic drugs. If severe, inject diphenhydramine, 50–100 mg intravenously or intramuscularly. For milder symptoms, oral antihistaminics are satisfactory.

F. Other Measures: Oxygen therapy must be given to correct hypoxia caused by respiratory obstruction or shock. Cardiopulmonary resuscitation should be administered in case of cardiac arrest. Corticosteroids have no known "antianaphylactic" property, and there is no rationale for their use in the critical initial stages of treatment. However, they may be helpful in refractory shock, persistent urticaria or angioedema after subsidence of the acute reaction, and bronchospasm in the asthmatic patient previously treated with corticosteroids.

The management of anaphylaxis from a Hymenoptera sting is the same as for any anaphylactic reaction (see above). In the case of honeybee sting, the venom sac and stinger usually remain in the skin and should be removed promptly by scraping with a knife or fingernail. Local reactions usually require only cold compresses to ease pain and reduce swelling, but extensive local inflammation may require brief corticosteroid therapy.

Prevention

Once an episode of anaphylaxis has occurred, every effort should be made to identify the allergen so that the patient can avoid further exposure. Any physician or nurse who administers drugs by injection should be prepared to treat a possible anaphylactic reaction by having appropriate drugs available, and patients should remain under observation for 15–20 minutes after any injection.

A. Hyposensitization: The effectiveness of immunotherapy in Hymenoptera venom anaphylaxis is now accepted on the basis of recent clinical studies. Patients with systemic anaphylactic reactions by history and positive skin test to the corresponding Hymenoptera venom should receive injection therapy using the specific venom. Injections of whole body insect extracts are ineffective.

B. Anaphylaxis Kit: The patient should carry at all times a small kit containing a preloaded syringe of epinephrine and an antihistamine tablet. Epinephrine or isoproterenol in a pressurized-aerosol hand nebulizer is not a reliable means of protection for anaphylactic shock.

C. Protective Measures: Avoid using strong scents such as perfumes and hair sprays when outdoors since these attract insects. Wear shoes outdoors. Avoid garbage cans. Do not tamper with beehives or with wasp or hornet nests.

Complications & Prognosis

Death from laryngeal edema, respiratory failure, shock, or cardiac arrhythmia usually occurs within minutes after onset of the reaction, but in occasional cases irreversible shock persists for hours. Permanent brain damage may result from the hypoxia of respira-

tory or cardiovascular failure. Urticaria or angioedema may recur for months after penicillin anaphylaxis.

URTICARIA & ANGIOEDEMA

Urticaria affects many people at some time, usually as an acute self-limited episode but occasionally in a chronic or recurrent form. The lesion—a localized area of increased vascular permeability—appears as multiple areas of well-demarcated swelling of the skin, usually accompanied by pruritus. It can result from a variety of causes, some of which are immunologic. Angioedema is a similar condition in which the affected blood vessels are deeper, resulting in diffuse swelling, usually without pruritus. Urticaria and angioedema may appear together in the same patient. When an allergic cause can be found, the disease is actually a localized cutaneous form of anaphylaxis, since the immunologic mechanism and the causative allergens are similar.

Etiology

Allergy, infections, physical factors, certain systemic diseases, and emotional stress have all been associated with urticaria.

Ingestant allergens are much more frequent causes of urticaria than are inhalants. Any food or drug can cause hives. Occult sources of drugs such as penicillin in milk and the use of proprietary medications such as laxatives, headache remedies, and vitamin preparations must be considered. Food and drug additives are occasionally responsible. Aspirin and nonsteroidal anti-inflammatory drugs cause urticaria and angioedema by a nonimmunologic mechanism, so they should be avoided by patients with chronic idiopathic urticaria.

A localized or systemic infection may provoke urticaria as part of an immune response to the infecting organism. This is particularly true with parasitic diseases, which are often associated with eosinophilia and a prominent IgE immune response. Urticaria may appear during the prodromal phases of certain viral infections, especially hepatitis B and infectious mononucleosis. Bacterial infections are much less likely to cause urticaria.

"Physical allergy" refers to a type of urticaria in which external physical stimuli cause hives or swelling. The most frequent type is cold urticaria, in which exposure to cold temperature results in hives and angioedema, either during the cold exposure or after rewarming. In some cases, the sensitivity to cold can be passively transferred by serum to normal skin, suggesting an antibody mechanism, but the nature of the antigen is unknown. It is assumed that cold alters a normal skin protein in such a way as to make it antigenic. Occasional patients have been described with urticaria or angioedema on exposure to heat, sunlight ("actinic"), water ("aquagenic"), and vibratory stimulation of the skin. The localization of urticaria to areas of the skin subjected to mild pressure or trauma is characteristic of urticaria in general.

Cholinergic urticaria is a condition in which small wheals with a large area of surrounding flare appear after exercise, from exposure to an overheated atmosphere, or during emotional stress

Urticaria occasionally is a sign of an underlying systemic disease. Neoplasms—especially Hodgkin's disease and lymphomas—and connective tissue disorders—particularly systemic lupus erythematosus—have been reported to cause hives.

Emotional trauma can precipitate acute urticaria or angioedema or aggravate the chronic form of the disease.

Immunologic Pathogenesis

Several different mechanisms are capable of causing increased cutaneous vascular permeability expressed as urticaria and angioedema. IgE antibodies to foods, drugs, or insect venoms sensitize cutaneous mast cells for release of histamine, which results in acute hives on exposure to the allergen. IgG or IgM antibodies complexed to antigen—or aggregated immunoglobulin without antigen—may activate the classic pathway of complement, generating anaphylatoxins C5a or C3a, which can stimulate mast cells for mediator release. Activation of the alternative complement pathway might also generate these anaphylatoxins, since they arise from the final effector pathway in the complement sequence. However, the clinical relevance of complement-dependent mechanisms in urticaria is uncertain. Physical agents or trauma might release histamine from mast cells by mechanical stimulation. Aspirin and nonsteroidal anti-inflammatory drugs have a nonspecific potentiating effect on urticaria and angioedema, suggesting a possible role for the leukotrienes which are products of the lipoxygenation of arachidonic acid.

Immunologic Diagnosis

As in any allergic diagnosis, a thorough medical history and complete physical examination are essential in order to interpret the significance of any subsequent test. Skin testing with the usual inhalant allergens is warranted only in those unusual cases in which the history suggests a direct causal relationship of such allergens to the patient's urticaria.

Food allergy is diagnosed by careful dietary history, use of elimination diets, and appropriate food challenges. Drug allergy requires close scrutiny of the patient's recent drug history, elimination of suspected drugs, and occasionally deliberate challenge, although skin testing is helpful for certain drugs such as penicillin. The diagnosis of cold urticaria is made by application of an ice cube to the forearm for 5 minutes and the appearance of localized urticaria after the skin has been rewarmed. Similar tests with heat, ultraviolet light, or water to a test area of skin are appropriate if the history suggests these causes.

Diagnostic tests for parasitic or other infections, lymphomas or other neoplasms, or connective tissue diseases are generally indicated only if the history and physical examination would have suggested such dis-

eases in the absence of urticaria. It should be emphasized that in most cases of chronic recurrent urticaria, no cause is found even with the most diligent search.

Differential Diagnosis

Multiple insect bites may evoke wheals, but careful inspection will show the bite punctum at the center of the lesion. Angioedema can be distinguished from ordinary edema or myxedema by its absence from dependent areas of localization and its evanescent appearance.

Treatment

Urticaria caused by foods or drugs is treated by avoidance of the offending agents, although hyposensitization to a drug might be attempted in rare instances in which no alternative drug is available. Urticaria associated with infection is self-limited if the infection is adequately treated. In cases of physical allergy, protective measures to avoid heat, sunlight, or cold must be advised.

Drug treatment is a useful adjunct in the management of all patients whether or not the cause has been found, but a good response to symptomatic treatment should not deter the physician from efforts to find an underlying cause. Antihistaminic drugs are the principal method of treatment, but they must be given in adequate dosage. Epinephrine injections may relieve hives transiently and should be used in treating angioedema involving the pharynx or larynx. Corticosteroids are usually ineffective and should not be used to treat urticaria of unknown cause.

Hereditary Angioedema

This is a rare form of angioedema inherited as an autosomal dominant deficiency of C1 inactivator (C1 esterase inhibitor). The defect produces an uncontrolled activation of the early components of the complement system, with generation of a kininlike substance in the plasma causing recurrent episodes of gastrointestinal and genitourinary tracts, and the larynx. Urticaria does not occur. Death can result from laryngeal angioedema.

The history is usually sufficient to suggest the diagnosis, which can then be confirmed by demonstrating a markedly diminished serum C4 even during symptom-free intervals. Levels of other complement components are usually normal. The diagnosis is established by measurement of C1 inactivator protein by immunoassay and its functional activity by hemolytic assay. There are 2 variants of the disease: in about 85% of kindreds, the C1 inactivator is absent from the serum, and in the remaining 15% it is present in normal amount but is functionally inactive. The disease was once thought to be extremely rare, but the availability of a specific laboratory diagnosis has made possible identification of a significant number of affected families.

Several modes of treatment have been advocated. Plasmin inhibitors such as aminocaproic acid (EACA; Amicar) orally have been helpful in preventing or ameliorating attacks in some patients, possibly by inhibiting fibrinolytic generation of the active kinin peptide. Androgen therapy using methyltestosterone or attenuated sex hormones such as oxymetholone and danazol not only prevents attacks but significantly increases the serum concentrations of C1 inactivator and C4, suggesting that these hormones may correct the inherited deficiency. Administration of fresh plasma to supply the missing inactivator has also been advocated to prevent attacks that might be precipitated by trauma such as dental surgery, but this approach has not been evaluated critically. Any attack of laryngeal edema occurring in a patient with this disease demands close observation—even hospitalization if necessary—in case tracheostomy is required.

Rarely, acquired C1 esterase inhibitor deficiency and angioedema can arise in a patient with lymphoma. Unlike the hereditary form of the disease, serum levels of C1 as well as C4 are low.

SERUM SICKNESS

Major Immunologic Features

- Serum sickness is a systemic type III immune complex complement-dependent reaction to an extrinsic antigen.
- Severity is antigen dose-dependent.
- The typical reaction produced by heterologous serum can occur in milder forms from other drugs.

General Considerations

Serum sickness was a common disease when heterologous antiserum was used as passive immunization in the treatment of a number of infectious and toxic illnesses in the preantibiotic era. "Serum therapy" using foreign (usually equine) serum or gamma globulin today is restricted to a very few toxic diseases (see Chapter 37) and the use of antilymphocyte or antithymocyte globulin for immunosuppressive therapy. Other drugs may occasionally cause a mild serum sickness reaction, especially sulfonamides, penicillin, and cephalosporins.

Immunologic Pathogenesis

The classic studies on "one-shot" serum sickness in rabbits by Germuth and Dixon clarified the pathogenesis of the human disease, to which it appears to be similar immunologically. A high-dose protein antigen exposure elicits a brisk IgG or IgM antibody response. When newly formed antibody reacts with residual antigen in the circulation to form immune complexes in moderate antigen excess, the circulating immune complexes are deposited on vascular endothelium in various organs. Activation of complement via the classic pathway generates chemotactic factors that localize inflammatory cells in and around blood vessels. Failure of mononuclear phagocytes to adequately clear immune complexes is probably an important factor in

disease expression. If further antigen exposure is avoided, the disease is self-limited, because complement activation is much less efficient in the presence of antibody-excess complexes. Some manifestations of the disease may relate to activation of complement-derived anaphylatoxins, vasoactive peptides, or coincidental IgE antibody response.

Clinical Features

The symptoms of serum sickness begin usually 7–14 days after exposure to the allergen. Fever and malaise are common. Arthralgias occur frequently, but they are generally mild to moderate, involving the large joints of the extremities. Clinical evidence of synovitis and periarticular inflammation is unusual. Skin lesions are common and may take the form of urticaria or a generalized morbilliform eruption beginning over the trunk and spreading peripherally. Lymphadenopathy and mild gastrointestinal symptoms are sometimes present. Transient proteinuria may occur without glomerulonephritis (in contrast to the rabbit model).

Immunologic Diagnosis

The history of typical symptoms after exposure to antiserum or drug is often sufficient for a definitive diagnosis. During the symptomatic phase, circulating immune complexes can be detected especially by the C1q binding method. C3 and C4 levels are diminished. After days or weeks, all clinical signs and laboratory abnormalities return to normal.

Treatment

Therapy is symptomatic only. Antihistamines will relieve the itching associated with the skin lesions, and aspirin can be used for arthralgias and fever. While corticosteroids might logically be used to inhibit inflammation, a recent study showed that almost all patients given antithymocyte globulin developed serum sickness in spite of pretreatment with high-dose steroids.

Complications

While the disease is usually self-limited and subsides completely, occasional neurologic involvement, especially mononeuritis multiplex, may occur.

ALLERGIC VASCULITIS

There are a group of diseases characterized by inflammation of blood vessels, usually arteries although both arteries and veins may be affected in some cases. The diseases include polyarteritis nodosa, Wegener's granulomatosis, Kawasaki disease, Churg-Strauss syndrome, hypersensitivity angiitis, and Henoch-Schönlein purpura. The unifying feature of these diseases is systemic vasculitis, but each is distinguished by characteristic pathologic features which determine many of the clinical findings that define the specific syndromes. Variables include the size of the affected vessel, the involved organs, the presence or absence of vascular necrosis, and the type of inflammatory reaction, especially the presence or absence of granuloma.

Vasculitis is a frequent accompaniment to inflammation, whether induced by infection (eg, subacute bacterial endocarditis), hypersensitivity (eg, serum sickness), autoimmune disorders (eg, systemic lupus erythematosus, rheumatoid arthritis), or cancer. The other diseases listed above, however, occur without known cause. The apparent primary nature of the vasculitis and the occurrence of overlap syndromes favor a single etiology and pathogenesis at this time.

An allergic origin, patterned mainly after the serum sickness model, is suggested—at least in some of the systemic vasculitides—by the presence of circulating immune complexes and evidence of complement consumption in the blood with deposition in tissues, the known pathogenic effect of immune complexes in vasculitis, and the occurrence of disease in some patients after administration of drugs, especially sulfonamides, thiazides, and phenytoin. Eosinophilia and a history of allergy and asthma are present in some cases, suggesting a role for IgE hypersensitivity or complement-generated anaphylatoxins. Granulomatosis, on the other hand, is consistent with T cell-mediated hypersensitivity. If indeed an exogenous cause (allergen) is involved in some or all cases of systemic vasculitis, individual differences in the quantity and quality of the immune response by the host could explain the presence of a particular syndrome. A careful search for possible antecedent exposure to allergens—especially drugs—is warranted in evaluation of each case.

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Gastrointestinal & Liver Diseases 25

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Gut-associated lymphoid tissue (GALT) of the mammalian alimentary tract and liver is, quantitatively, a major component of the total immune system of the body. It is essential for homeostasis in the alimentary tract and contributes to immune events in mucosal structures of other organs of the body.

The lymphoreticular components of the mature gastrointestinal tract are categorized, by morphologic and functional criteria, into 3 populations: Peyer's patches, intraepithelial lymphocytes, and a mixed population of lymphocytes, plasma cells, macrophages, and mast cells in the connective tissue space, or lamina propria, of intestinal mucosa, which lies deep to the epithelium.

STRUCTURAL & FUNCTIONAL COMPONENTS OF THE IMMUNE SYSTEM OF THE GASTROINTESTINAL TRACT

Peyer's Patches

These are aggregates of subepithelial lymphoid follicles located in the mucosa of the small intestine. They are more numerous in the ileum than the jejunum. In humans they appear by the 24th week of gestation and become more numerous and larger until the completion of puberty. Each patch is only one follicle thick. The overlying epithelium is devoid of villi and consists of specialized **microfold (M) cells.** These are believed to provide a pathway by which antigens in the lumen of the gut are brought into contact with macrophagelike cells in Peyer's patches. These process and present some antigens to lymphoid cells, which are thereby activated. Serial studies in animals have shown that Peyer's patches in early stages are populated chiefly by thymus-derived T cells. Marrow-derived B cells increase in number subsequently, so that in adult animals the ratio of T cells to B cells is of the order of 70:30. Both circulating B and T lymphocytes enter Peyer's patches by a special mechanism of binding to the high-endothelial cells of venules in the patches. Choice of a Peyer patch—or some other type of peripheral lymph node—appears to reside in specific lymphocyte surface components that have been identified antigenically. The distribution of T and B cells in the mature Peyer patch is not random. The lymphoid follicles contain B cells predominantly, on the surface of which IgA is present. The interfollicular areas contain most of the T cells.

Antigen activation occurs in Peyer's patches and depends on class-specific helper T cells that transform IgM to IgA lymphoblasts. Activation results in proliferation, exit from Peyer's patches into the circulation, and migration to the intestinal epithelium, the lamina propria, and also to the bronchial and genitourinary tracts and to other structures such as the mammary, salivary, and lacrimal glands. Bronchial mucosal lymphoblasts apparently migrate reciprocally to the intestinal mucosa. These migrations have been demonstrated for B cells, and it is currently believed that T cells behave similarly. The immunoglobulin secretory response of B cells is T cell-dependent, and lack of IgA class-specific helper T cells may be responsible for intestinal IgA deficiency.

Intraepithelial Lymphocytes (Thellolymphocytes)

These are predominantly T lymphocytes (> 90%), and the majority (> 80%) express the phenotype of the suppressor-cytotoxic T cell subpopulation (CD8). They lie in close relationship with intestinal epithelial cells and constitute possibly one-third of the total lymphoid tissue of the intestine. The function and fate of these cells, which may not constitute a homogeneous population, is not known. The majority do not persist for more than 3–4 days in the epithelium. Some are shed into the intestinal lumen and may assist in the destruction of pathogenic protozoa, eg, *Giardia*.

Lymphoreticular Cells of the Lamina Propria

Plasma cells are distributed rather homogeneously throughout the lamina propria of the large and small gut and in much smaller numbers in the lamina propria of the stomach. They are not present before birth, and their appearance coincides with population of the alimentary tract with microorganisms. It has been shown experimentally that sterile fetal small bowel implanted autologously under the skin or renal capsule will also become populated with plasma cells, demonstrating that exogenous antigens may not be necessary for this phenomenon to occur. Plasma cells are derived from large B lymphocytes. They contain immunoglobulins of classes A, G, M, E, and D. IgA-containing cells predominate (A, 80%; M, 15%; G, < 5%; D and E, 2%) and contribute part of the total circulating 7S IgA. IgA-containing cells occur in higher proportion in small than in large intestine, and the reverse is true of IgG cells. Approximately 60% of IgA- and IgM-con-

taining cells are found within 200 μm of the surface epithelium; the majority of IgG-containing cells are found more basally. In addition to mature plasma cells and B lymphocytes, there is a large population of T cells, also derived from Peyer's patches. Studies with monoclonal antibodies have shown these to be helper/inducer T cells (CD4). There are also large numbers of Ia-bearing macrophages in the lamina propria, which have the potential of serving as antigen presenters.

IMMUNOGLOBULINS IN THE INTESTINAL TRACT (See Chapter 12.)

All classes of immunoglobulin are found in gastric and intestinal secretions, but IgA predominates. The proportion of subclass IgA2 to IgA1 is more than 50% in secretions, compared with about 10% in serum. Similarly, immunofluorescence studies reveal all classes of immunoglobulins in the intercellular spaces of the lamina propria, but IgG predominates.

The IgA in gastrointestinal (and other) secretions is largely in the form of an 11S dimer designated secretory IgA (sIgA). This differs from the predominant type of IgA in serum by the addition of a so-called J piece, a polypeptide of about MW 15,000, which appears to be necessary for the dimeric IgA to combine with a glycoprotein of approximately MW 60,000 called secretory piece or secretory component (SC) (Fig 25–1). SC is synthesized within the endoplasmic reticulum of the epithelial cells of the mucosa, transported to the Golgi complex, where it is found in unbound form, and then acts as a receptor, at the basal surface of the epithelial cell, for the dimeric J chain-containing IgA. The resulting complex is taken into the epithelial cell by pinocytosis, passes across the cell, and is discharged into the intestinal lumen. Not only dimers but also small amounts of trimers and larger polymers of IgA may be present in secretions. IgM is also secreted by plasma cells as a J chain-containing polymer and is transported similarly to secretory IgA, being bound to SC. In intestinal secretions, more than two-thirds is present as secretory complex.

Secretory IgA therefore contains 4 types of polypeptide chains: four L, four H, one J, and one SC. It is more resistant than 7S IgA to proteolysis. It possesses specific antibody activity, and its presence is associated with blocking of adherence of some microorganisms to epithelial cells. Both 7S and secretory IgA activate complement by the alternative pathway and are not, therefore, involved in Arthus-type reactions.

Secretory IgA constitutes the major part of the coproantibodies found in the feces of humans and animals following natural, therapeutic, or experimental infection, including immunization. Similar antibody production in response to ingested antigens unrelated to microorganisms and to autoantigens of the gastrointestinal tract itself has been demonstrated. Immunoglobulin-containing plasma cells in the lamina propria of stomach or gut have been shown to contain specific antibodies to such antigens, and IgA predominates. It is therefore clear that the gut is capable of mounting a local primary immune response. Such a response could be (1) protective, (2) regulatory of the gut flora, or (3) pathogenic.

In humans and other species, it has been shown that oral administration of antigen also results in the production of **circulating** antibodies. The class of serum antibodies formed as a consequence of oral administration of antigen varies. In mice fed ferritin, it is predominantly IgA, whereas in humans administration of live attenuated poliovirus is followed by a response initially of IgM followed by IgG, similar to that seen after parenteral inoculation. Systemic lymphoid tissue acquires the capacity to produce antibodies to

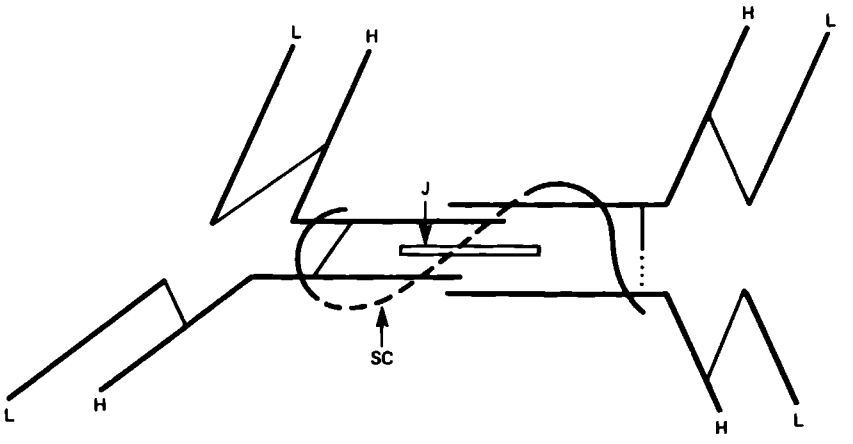

Figure 25–1. Model for secretory IgA. SC = secretory component; H = heavy chain; L = light chain; J = chain. (Modified from Heremans JS: The IgA system in connection with local and systemic immunity. In: *The IgA System*. Mestecky J, Lawton AR [editors]. Plenum Press, 1974.)

ingested antigens. Thus, antigen must reach this lymphoid tissue across the mucosal and vascular structures, or lymphoid cells that are committed to specific antibody production migrate to systemic lymphoid tissue from the gut.

THE GASTROINTESTINAL TRACT AS A SITE OF IMMUNOLOGIC REACTIONS

Theoretically, the gastrointestinal tract might be involved in both immediate and delayed immunologic reactions.

Anaphylactic responses occur as a consequence of reaction of dietary allergens and specific IgE (reagin) bound to Fc receptors of mast cells in the gut wall. Subacute or chronic tissue damage may occur as a consequence of antibody-dependent and -independent cytotoxic reactions, immune complexes activating the complement system, or sensitized T lymphocytes interacting with antigens and releasing lymphokines.

Gut-Induced Immunologic Tolerance

A vast array of extrinsic antigens, microorganisms, and dietary constituents is presented to the gastrointestinal mucosal surfaces. Many mechanisms exist whereby ingested antigens induce a state of antigen-specific systemic hyporesponsiveness or tolerance, though there may be a concomitant local mucosal secretory response. In some instances, specific suppressor T cells, produced in Peyer's patches, appear to be responsible. In others, deletion of helper T cells may occur. Other possible mechanisms are the production of "blocking" antibodies to the ingested antigen, or of low concentrations of IgA antibody-antigen complexes, formed in antigen excess, acting as tolerogens. The intestinal wall and the liver may share the ability to mediate immunologic tolerance, since injection of some antigens into the portal vein produces a similar state and bypassing of the liver by portal-systemic venous anastomosis abolishes the ability of ingested antigens to induce the tolerant state.

In summary, the mechanisms of induction of tolerance by antigen ingestion depend on the nature, dose, and duration of administration of the antigen, as well as the age, species, and genetic makeup of the recipient. The consequences of this phenomenon, however produced, must be profound.

ACUTE GASTROINTESTINAL ALLERGY

Major Immunologic Features

- Involves reaction of specific IgE antibody and alimentary antigens in some cases.
- Subsequent release of histamine and other mediators of immediate hypersensitivity.

General Considerations

Acute and subacute allergic reactions occurring in the gastrointestinal tract as a consequence of ingestion of allergens are well documented. It has been estimated that in the USA 0.3–0.7% of infants are sensitive to cow's milk protein. The β-lactoglobulin component is an important allergen. It is absent from human milk, which usually does not evoke a reaction in an infant intolerant to cow's milk. Tolerance develops with maturity in most subjects. Hypersensitivity to other dietary proteins occurs less frequently. What may be interpreted as allergy to milk is sometimes an expression of intestinal lactase deficiency and lactose intolerance. This may be primary, or secondary to intestinal damage associated with an allergic response. Reactions to small amounts of milk or to lactose-free milk products such as cheeses are not due to alactasia and are probably allergic.

Immunologic Pathogenesis

The reason that some individuals are atopic is unknown. The atopic child appears to have the same exposure to ingested antigens as the normal child. Some data suggest that hypersensitivity occurs significantly more often in subjects who are deficient in IgA. Absence of secretory IgA in the intestinal mucosa or lumen may impair the physiologic mechanisms responsible for excluding potential allergens from the body without involvement of tissue-damaging or exciting reactions. The consequence might be the recruitment of more vigorous immune reactions involving other classes of antibody. Stimulation of normally responsive precommitted IgE plasma cells during a transient IgA-deficient period at the age of about 3 months has been proposed as a cause of the development of atopy to dietary proteins. An apparently—though not necessarily—conflicting view is that acute allergic disease occurs as a consequence of early contact with allergens and a genetically favored IgE pathway of antibody formation.

The sequence of events following presentation of an allergen to the intestinal tract has been studied by serial mucosal biopsy in infants sensitive to cow's milk. At 6 hours after challenge, edema and a small excess of plasma cells are noted in the jejunal lamina propria. By 12 hours, the numbers of mast cells and plasma cells, eosinophils, and neutrophils have greatly increased. The increase of plasma cells consists mainly of those containing IgE and IgM.

Experimental studies of passive anaphylaxis in intestine and skin have shown that the magnitude and timing of responses at both sites are the same whether the antigen is administered orally, intravenously, or locally. This suggests that allergen does not reach mast cells in the mucosa directly from the lumen but via the bloodstream. Thus, inhaled or injected allergens may produce reactions in the intestinal tract.

Clinical Features

A. Symptoms and Signs: Abdominal discomfort, nausea and vomiting, watery and sometimes bloody diarrhea, and, in severe cases, prostration and fever may occur. Extraintestinal responses such as bronchospasm, skin eruptions, and migrainous

headaches may complicate or dominate the clinical picture. Chronic gastrointestinal reactions to cow's milk are seen much more frequently than acute gastrointestinal allergy. The former occur in infants 2–3 months old, typically 1 month after introduction of cow's milk into the diet. They are characterized by vomiting, prolonged diarrhea, failure to thrive, and sometimes atopic asthma and recurrent respiratory infection.

B. Laboratory Findings: Transient or persistent eosinophilia may be the only objective finding. In clinical practice, the diagnosis often depends solely on the history, and objective evidence is rarely obtained. Lacking evidence, many clinicians regard the whole subject skeptically.

Immunologic Diagnosis

The results of skin tests with suspected allergens correlate poorly with the clinical history. The presence of IgG and IgM serum antibodies to dietary proteins detected by standard techniques such as gel diffusion and passive hemagglutination does not correlate positively with clinical sensitivity.

Studies by Ishizaka and others have established that reagins are immunoglobulins of the class designated IgE. IgE-containing plasma cells are found in relatively small numbers in the mucosa of the small intestine, which may be the site of sensitization to dietary allergens. Increased numbers of IgE plasma cells have been reported in the jejunum of children with food allergy. Elevated serum IgE concentrations are found in some but not all subjects with gastrointestinal allergy. Other antigen-specific immunoglobulin classes or subclasses, such as IgG4 or IgD, may be involved.

The Prausnitz-Küstner (PK) test, which requires intradermal injection of the possibly allergic subject's serum into a healthy human recipient followed by injection of the suspected antigen at the same site, provides good evidence of specific circulating IgE antibodies, but the test is positive in only two-thirds of subjects with unequivocal acute gastrointestinal and alimentary allergy. The risk of transmitting viral hepatitis has eliminated the use of this test in humans. The response can be elicited in other primates.

Confident diagnosis of gastrointestinal allergy is difficult. Tests other than the PK are required. Human skin from cadavers has been used successfully in vitro as a site for the anaphylactic reaction. It is unlikely to be more discriminating than the PK test. Radioallergosorbent techniques may provide sensitive methods for detecting specific reagins with variable concordance with provocative tests. At present, tests involving dietary exclusion and challenge, sometimes combined with jejunal biopsy, are the diagnostic tools most often employed. There is a need for additional valid double-blind tests, since there is often a psychogenic component to the complaints of sufferers from gastrointestinal allergy.

Differential Diagnosis

Acute gastrointestinal allergy may be mistaken for lactase deficiency, infectious gastroenteritis, or the onset of chronic nonspecific ulcerative colitis. Persistence of symptoms despite exclusion of cow's milk or other protein components of the diet prior to the clinical attack points to some other cause of the symptoms and requires further evaluation.

Treatment

Appropriate clinical tests, including fecal examinations and proctosigmoidoscopy, should be done before embarking on exclusion diets. Treatment consists of identifying the allergen and excluding it from the diet. If it is an important item nutritionally, a substitute must be found.

Trials of cromolyn sodium, administered orally to atopic children, have been encouraging.

Complications & Prognosis

Complications of an acute attack are vomiting and diarrhea, resulting sometimes in serious dehydration and circulatory collapse. Bronchospasm may rarely be fatal, as may acute laryngeal edema.

Malabsorption and failure to thrive, atopic eczema, and recurrent respiratory infections are seen in less severely affected children until the specific allergen is excluded from their diet. Available data suggest that the hypersensitivity abates with age in most children and that in some cases full recovery is possible.

CHRONIC OR RELAPSING INFLAMMATORY DISEASES OF THE GASTROINTESTINAL TRACT

1. APHTHOUS ULCERATION OF THE BUCCAL CAVITY

The lesions consist of painful, recurrent ulcers on the tongue and buccal mucosa, lasting several days. About one in 10 otherwise healthy subjects suffers from the condition. In some, exposure to chocolate, nuts, or other foodstuffs is the precipitating cause. The lesions begin as tender red swellings and on histologic examination show acute inflammatory change with some mononuclear cell infiltration. The surface sloughs and becomes secondarily infected; healing occurs over days or weeks, usually leaving no scar.

The pathogenesis is unknown. The disease is not associated with herpes simplex. In some patients, a specific allergen can be identified. Serum antibodies to crude extracts of buccal mucosa have been demonstrated by complement fixation in subjects suffering from recurrent aphthous ulceration, although their significance is uncertain.

In sprue, Crohn's disease, ulcerative colitis, and intestinal lymphoma, more severe aphthous ulceration may occur, causing anorexia and providing a site for candidal and other infection. It is not known whether this more severe type of lesion is an expression of poor nutrition or of an abnormal immune response, possibly of an immune complex disease, as has been sug-

gested in the case of pyoderma gangrenosum associated with inflammatory bowel disease. The lesions respond when the bowel disease is treated successfully.

2. SJÖGREN'S SYNDROME (See Chapter 21.)

The complete Sjögren's syndrome consists of conjunctival and buccal dryness, recurrent swelling of lacrimal and salivary glands, rheumatoid arthritis, and a higher incidence of malignant lymphoma than in the normal population. Salivary glands show plasma cell and lymphocyte infiltration.

Humoral antibodies to salivary tissue occur; in a large proportion of patients, antibodies to thyroid and nuclear antigens may also be found (Table 25–1).

3. CHRONIC ATROPHIC GASTRITIS & PERNICIOUS ANEMIA

Major Immunologic Features

- Evidence for both humoral and cellular immunity to gastric mucosal antigens.
- High incidence of anti-parietal cell antibodies.
- Autoantibodies found in gastric mucosal plasma cells and in secretions (IgA).
- Parietal cell cytotoxicity.

General Considerations

The primary pathologic feature of pernicious anemia is progressive destruction of the normal glands of the body of the stomach. This is associated with partial or complete loss of the chief cells, which secrete pepsinogens, and the parietal cells, which secrete acid and intrinsic factor. When the last is complete, failure of vitamin B_{12} absorption occurs. Complete loss of intrinsic factor secretion may require many years or may never occur. The gastric mucosa is infiltrated, homogeneously or in patchy distribution, with variable numbers of polymorphonuclear and mononuclear leukocytes—predominantly the latter. Often the gastric body mucosa is partly or wholly replaced by antral or small intestinal mucosa, a phenomenon known as metaplasia.

Immunologic Pathogenesis

Experimentally, chronic inflammatory lesions of the gastric mucosa, glandular atrophy, and loss of gastric secretory function have been produced in dogs by immunization using gastric mucosal material and Freund's complete adjuvant. Other species, such as rabbits, rats, mice, and guinea pigs, are resistant to these procedures. In dogs, the appearance of gastric lesions is associated more with development of cell-mediated immunity than with humoral antibodies. However, repeated injection of human gastric antibodies into rats has reduced gastric secretory cells after several weeks.

In pernicious anemia, humoral antibodies are found to 3 antigens of the gastric parietal cell. The 2 most thoroughly characterized are a glycoprotein called Castle's intrinsic factor and a lipoprotein of the microvilli of the parietal cell canalicular system. The third, which appears to be distinct from the latter, is found in the surface membrane of the parietal cell. All are cell-specific. Serum parietal cell canalicular antibody is present, as determined by immunofluorescence, in about 90% of patients with pernicious anemia, and intrinsic factor antibody in 60–70%.

There are 2 types of intrinsic factor antibody: type I blocks the attachment of vitamin B_{12} to the intrinsic factor molecule, and type II attaches to intrinsic factor or the intrinsic factor-B_{12} complex (Fig 25–2). Type II intrinsic factor antibody occurs with about half the frequency of type I and only rarely occurs in the absence

Table 25–1. Autoantibodies in gastrointestinal and hepatic disease.

Disease	Antigen	Detection of Antibody
Aphthous ulceration	Buccal mucosa	Complement fixation
Sjögren's syndrome	Salivary ducts	Immunofluorescence
	Nuclei	Immunofluorescence and complement fixation
	Thyroid	Immunofluorescence and complement fixation
Pernicious anemia	Parietal cell canalicular lipoprotein	Immunofluorescence on gastric mucosa
		Complement fixation
	Parietal cell membrane	Immunofluorescence
	Thyroid	Immunofluorescence and complement fixation
	Intrinsic factor	Inhibition of biologic activity
		Blocking B_{12} binding
		Coprecipitation
Chronic antral gastritis	Gastrin-secreting G cells	Immunofluorescence
Gluten-sensitive enteropathy	Reticulin	Immunofluorescence
Chronic inflammatory bowel disease	Colonic epithelial lipopolysaccharide	Immunofluorescence
		Passive hemagglutination
Chronic active hepatitis	Smooth muscle ("actomyosin")	Immunofluorescence
Primary biliary cirrhosis	Nuclei	Immunofluorescence
	Mitochondria	Immunofluorescence and complement fixation (kidney or other tissue)

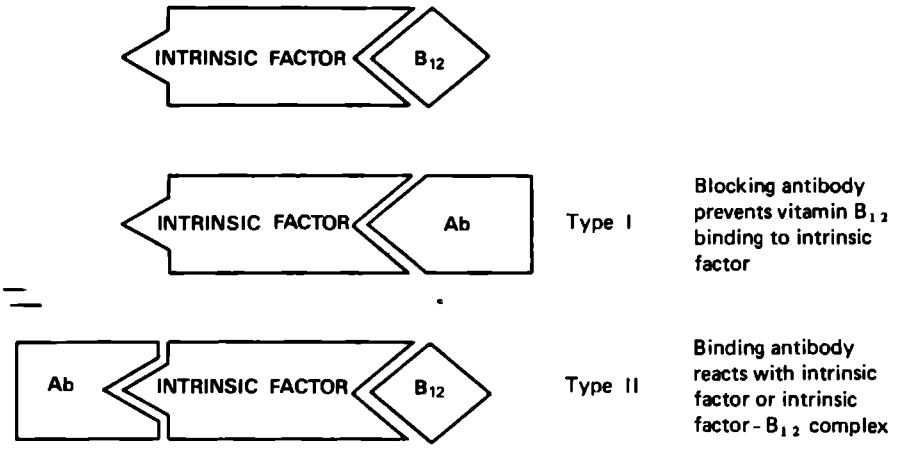

Figure 25–2. The 2 types of intrinsic factor antibody.

of type I. Experimentally, the immunogenicity of the intrinsic factor antigenic site for type I antibody is greater than that for type II. Evidence suggests that antibodies to intrinsic factor are capable of inhibiting intrinsic factor-mediated vitamin B_{12} absorption in vivo, and parietal cell canalicular antibody inhibits acid secretion by the parietal cell. Antibodies to the parietal cell surface, in the presence of complement, have recently been shown to possess specific cytotoxicity.

Gastric antibodies in serum are mainly of the IgG class, with some IgA and rarely IgM. In chronic atrophic gastritis of the body of the stomach without the lesion of pernicious anemia, serum parietal cell canalicular antibody occurs less frequently than in pernicious anemia, and intrinsic factor antibodies are found so rarely that their presence requires further diagnostic workup, which almost always reveals pernicious anemia. They rarely occur in the absence of severe gastritis, but a group of patients with thyroid disease has been described in which humoral intrinsic factor antibodies were present despite some acid and intrinsic factor secretion that persisted during many years of follow-up. Recently, serum antibodies to the gastrin-secreting G cells of the antral mucosa have been described in a small percentage of patients with chronic antral gastritis whose sera are negative for parietal cell canalicular or intrinsic factor antibodies.

Parietal cell canalicular antibodies—but not intrinsic factor antibodies— are found in some first-order relatives of patients with pernicious anemia. They are also present with significantly increased frequency in iron deficiency anemia, thyroid disease, idiopathic Addison's disease, diabetes mellitus, and vitiligo in association with chronic gastritis (Table 25–2). The infrequency of parietal cell canalicular antibody increases in apparently healthy subjects with age. This antibody is rarely or never found unless chronic gastritis is present, and the prevalence of this condition increases with age.

Thyroid microsomal and thyroglobulin antibodies are also found in some pernicious anemia subjects, though in lower frequency than in subjects with thyroid diseases.

Gastric autoantibodies are not detectable in patients with chronic gastritis associated with gastric and duodenal ulcer disease or following partial gastrectomy for ulcer disease, or in gastric carcinoma, except in subjects with preexisting pernicious anemia. In these, the presence or absence of circulating gastric antibodies apparently has no significance with regard to development of gastric carcinoma. The gastritic lesions in these conditions are histologically indistinguishable from those in pernicious anemia, but gastric functional changes are usually less severe.

Gastric antibodies in gastric secretions. Both parietal cell canalicular antibody and intrinsic factor antibody are present in the gastric juice of the majority of pernicious anemia patients. Intrinsic factor antibodies may form complexes with any residual intrinsic factor secreted. The antibodies are IgG and IgA, and some activity is present in 11S (secretory) IgA. This, together with demonstration of the presence of both parietal cell canalicular antibody and intrinsic factor antibody in plasma cells in the inflamed mucosa and the absence of thyroid antibodies from gastric juice in subjects with thyroid antibodies in their sera, attests to

Table 25–2. Frequency of circulating parietal cell canalicular antibody in pernicious anemia and other diseases.

Patient Group	Approximate Positive Parietal Cell Canalicular Antibody (%)
Pernicious anemia	86
Normal population*	11
Vitiligo	20
Thyroiditis	30
Thyrotoxicosis	25
Addison's disease	24
Diabetes mellitus	21
Iron deficiency anemia	20

*Chronic gastritis not excluded.

gastric antibody formation in the gastric mucosa. By immunofluorescence, complement fixation has been demonstrated at the site of parietal canalicular antigen when it reacts with antibody.

Cell-mediated immunity in pernicious anemia. Cellular immunity to intrinsic factor and gastric parietal cell canalicular antigen has been measured by lymphocyte activation and MIF production. Positive responses have been detected in patients with pernicious anemia, in patients with pernicious anemia and immunoglobulin deficiency, and in a few normal individuals.

Response of the gastric lesion of pernicious anemia to corticosteroids and immunosuppressive drugs. Improved vitamin B_{12} absorption, enhanced intrinsic factor secretion, some regeneration of gastric parietal cells, and decreased titers of circulating gastric antibodies have been reported in pernicious anemia subjects treated with corticosteroids. Achlorhydria usually persists. However, there is a lack of correlation in treated individuals between improved vitamin B_{12} absorption, improved gastric structure and function, and changes of intrinsic factor antibody titers in either serum or gastric juice, and the mode of action of corticosteroids remains uncertain.

Parietal cell regeneration and restoration of gastric acid secretion have been reported in patients with pernicious anemia treated with azathioprine, but no immunologic studies were performed. No direct effect of immune cells or of gastric antibodies on human gastric mucosa has been observed. Until this is achieved, evidence of an immunologic pathogenesis in pernicious anemia is only presumptive.

Clinical Features

A. Symptoms and Signs: Atrophic gastritis without pernicious anemia is frequently undiagnosed, since the gastric lesion is usually asymptomatic. Mild to severe dyspepsia may occur together with impairment of appetite. Pernicious anemia is the expression of vitamin B_{12} deficiency. It is characterized by increasing weakness, fatigability, loss of appetite, and pallor. Loss of weight is common, but wasting is rare. Involvement of the nervous system, which is quite variable, may include peripheral neuropathy, damage to pyramidal tract and posterior column neurons, and disturbance of higher cortical functions.

Pernicious anemia displays a strong familial association, but how much is environmentally and how much genetically determined is still unknown. No specific HLA genotype has been unequivocally associated with the disease. There is an association between pernicious anemia and thyroid disease, especially thyrotoxicosis, which occurs frequently in close relatives of pernicious anemia patients. Less strong associations have been claimed with diabetes mellitus and idiopathic Addison's disease. Pernicious anemia also occurs in acquired immunoglobulin deficiency, most commonly of IgA but sometimes of IgG or IgM also.

B. Laboratory Findings: Macrocytic anemia and hypersegmentation of the nuclei of the neutrophil granulocytes are the morphologic findings in the peripheral blood. Bone marrow aspirates show megaloblastosis. Serum vitamin B_{12} values below 120 pg/mL are found (normal, 200–1500 pg/mL). Serum lactate dehydrogenase activity is markedly elevated owing to excessive intramedullary destruction of red blood cells. The excretion of methylmalonate in the urine is increased. Vitamin B_{12} therapy corrects all of these abnormalities except that some damage to the central nervous system may be irreversible.

Immunologic Diagnosis

The finding of circulating parietal canalicular antibodies by complement fixation or immunofluorescence almost certainly indicates chronic gastritic disease, although negative serologic findings do not exclude this diagnosis. Antibodies to intrinsic factor indicate the lesion of pernicious anemia, with the rare exceptions of a few patients with thyroid disease. Cell-mediated immune tests are not yet generally available.

Differential Diagnosis

Clinically, other anemias and untreated myxedema and pituitary hypofunction may be confused with pernicious anemia. The finding of megaloblastic anemia reduces the possibilities to 3—namely, deficiency of vitamin B_{12}, deficiency of folic acid, or both. Unequivocal involvement of the nervous system points to the former. However, a deficiency of vitamin B_{12} may occur as a consequence of dietary deficiency (an unsupplemented vegetarian diet) or of malabsorption due to pancreatic or small intestine disease as well as a lack of intrinsic factor. Correction of vitamin B_{12} malabsorption by administration of intrinsic factor provides unequivocal evidence of the basic lesion of pernicious anemia. Impaired absorption of vitamin B_{12} is demonstrable by a variety of tests using tracer doses of radioactive cobalt-labeled vitamin B_{12} without and with intrinsic factor (eg, the Schilling test, in which urinary excretion of labeled vitamin B_{12} is the index of intestinal absorption). Aspirated gastric juice contains negligible intrinsic factor activity and no hydrochloric acid, even in response to powerful stimulants of secretion. The presence of serum antibodies to gastric antigens may provide confirmatory evidence.

Treatment

Intramuscular injections of vitamin B_{12} will maintain remissions in pernicious anemia. Corticosteroids and immunosuppressive agents have only been used experimentally. There is no practicable means at present of recognizing the gastric lesion sufficiently early to offer any hope of instituting therapy at a reversible stage of the disease.

Complications & Prognosis

The only important complication is gastric carcinoma, which is probably 3 times more common in patients with pernicious anemia than in the population at large. No immunologic or other test has yet been de-

vised which reliably identifies subjects prone to develop gastric carcinoma or provides early recognition of such a lesion.

GLUTEN-SENSITIVE ENTEROPATHY; CELIAC SPRUE

Major Immunologic Features

- Genetic association with HLA-B8, -Dw3, and -Dw7.
- Infiltration of jejunal lamina propria with lymphocytes and plasma cells when untreated.
- Possible local IgA deficiency with production of IgM antigluten antibodies.

General Considerations

This disease, also termed nontropical sprue and, in adults, idiopathic steatorrhea, is a disease of the small intestine characterized by functional disturbances causing malabsorption of food and nutrients and structural changes of partial or complete villous atrophy. The normally columnar jejunal epithelial cells become stunted and cuboid, and distortion of their microvilli occurs. The lamina propria, the volume of which is reduced, becomes infiltrated with inflammatory cells, of which the majority are mononuclear in type. In contrast, the ileal mucosa is relatively or completely normal.

Dicke showed more than 30 years ago that ingestion of wheat gluten was the causative factor, and the term gluten-sensitive enteropathy has now been adopted. α-Gliadin is the noxious component of gluten, and some polypeptide products of α-gliadin hydrolysis as small as MW 8000 have been shown to produce mucosal damage in susceptible individuals. Exclusion of gluten from the diet results in restoration of normal mucosal structure and function.

Immunologic Pathogenesis

There is no evidence to support the early theory that intestinal damage in gluten-sensitive enteropathy is due to lack of a mucosal peptidase and consequent accumulation of toxic products of partial cleavage of α-gliadin. Some evidence favors a genetically determined immunologic pathogenesis—ie, findings of abnormal T cell-mediated responses to α-gliadin in individuals possessing HLA-B8, -Dw3, and -Dw7 antigens—but complete proof is lacking. The frequency of HLA-B8 in celiac disease is 60–80%, compared with 20–25% in the control populations.

The humoral response, which results in the synthesis of IgA and IgM α-gliadin antibodies in the intestinal mucosa and their presence in the circulation, is established. What role such antibodies play in the pathogenesis of the intestinal lesion is unclear. There is the possibility of damage caused by an Arthus-type reaction in the intestinal mucosa. Oral challenge of treated celiac subjects with gliadin fractions results in eosinophilic infiltration of the proximal intestinal mucosa in 4–5 hours, polymorphonuclear infiltration and vascular endothelial swelling at 10–16 hours, and an increase of predominantly IgM plasma cells, the IgM being largely antibody-specific for gliadin. IgM immune complexes appear in the basement membrane, with variable local uptake of complement. Electron microscopic studies have shown concomitant destruction of basement membrane. Such changes do not occur in nonceliac controls.

Proponents of a cell-mediated immunologic pathogenesis emphasize the increase in number of theliolymphocytes, seen within 12–48 hours of gliadin challenge, and in their increased transformation to blast cells. They also cite the continuing increased mitotic proliferation and turnover of mucosal lymphocytes in untreated celiac patients.

Some in vitro studies, using mixtures of circulating T cells and of antigen-presenting cells prepared from monocytes (adherent cells) from celiac patients in remission and healthy controls, have shown that lymphocyte stimulation in the presence of α-gliadin fractions is identical, in contrast to the much reduced response using cells from untreated celiac patients. It is assumed that mucosal sequestration of gliadin-sensitive T cells has occurred in the untreated subjects, which is consistent with the observed relative and absolute reduction of circulating T cells in intestinal celiac disease, restored after response to gluten exclusion.

It is further postulated that the mucosal damage in untreated celiac disease, including the villous flattening, is an expression of T cell activation, which may induce mast cell release of inflammatory agents, as is found in animal models of allograft reactions and helminth infestation.

In the current state of knowledge it is not possible to decide which mechanism is likely to be the pathogenic one. It is possible that both the immune complex Arthus-type reaction and the T cell-mediated phenomena share in the mucosal damage. It remains to be seen why initial sensitization to gliadin occurs in celiac disease.

Clinical Features

A. Symptoms and Signs: Malabsorption is associated with stunting of growth in the child, loss of weight in the adult, various deficiency states, and greasy, bulky stools of high fat content. The disease expresses itself at any age but most frequently in childhood.

B. Laboratory Findings: In the untreated subject there may be electrolyte depletion, reflected in abnormally low serum potassium and calcium values. Hypoalbuminemia may reflect protein depletion. Anemia is often present and may be due to iron deficiency, folic acid deficiency, or both. Analysis of the stools reveals increased excretion of fat and nitrogen, and tests of absorption of glucose and xylose reveal malabsorption. The diagnosis is established on the basis of an abnormal jejunal biopsy and response to gluten exclusion.

Recent studies have revealed an association of the

disease with the HLA-B8 histocompatibility antigens, suggesting a genetic basis for the previously noted familial association of the disease. The frequency of HLA-B8 in celiac disease is 80% compared with 20–25% in the population at large. (HLA-A1 is also much more frequent, but this gene is associated with HLA-B8 as a consequence of linkage disequilibrium.) An association with the HLA-Dw3 and -Dw7 haplotypes has been demonstrated, and that with HLA-B8 is thought to be secondary. It has also been noted that a skin disease, dermatitis herpetiformis, may be associated with small bowel malabsorption and that the latter responds like uncomplicated gluten-sensitive enteropathy to a gluten-exclusion diet. HLA-Dw3 and -B8 occur in dermatitis herpetiformis associated with malabsorption in an abnormal frequency similar to that in gluten-sensitive enteropathy. Thus, as in the case of ankylosing spondylitis, tissue histocompatibility typing may have diagnostic value in the future.

Reticulin antibodies are found in the sera of 60% of untreated gluten-sensitive enteropathic children, 30% of gluten-sensitive enteropathic adults, and 25% of patients with dermatitis herpetiformis. Following treatment by gluten exclusion, the antibody titer may fall or disappear. There is no validated explanation for this phenomenon. The occurrence of similar reticulin antibodies in subjects with small bowel inflammation due to other causes (eg, Crohn's disease) suggests a nonspecific response. Others have claimed that gluten and reticulin share antigenic determinants. The problem has still to be resolved.

Immunologic Diagnosis

There are no clinically useful immunologic tests. The finding of circulating gluten antibodies has not been applied diagnostically, but the presence of reticulin antibodies has proved to be helpful in the diagnosis of celiac disease in children. Arthus-type reactions to intradermal injection of a subfraction of gluten known to be pathogenic have been reported in subjects with celiac sprue, control subjects being uniformly negative.

Differential Diagnosis

The list of causes of small intestinal malabsorption in both children and adults is very large. In infants and children, sensitivity to cow's milk or dietary proteins other than gluten, lactase deficiency, cystic fibrosis of the pancreas, parasitic infestations, Crohn's disease, and congenital anatomic abnormalities of the gastrointestinal tract must be considered. In the adult, the consequences of upper gastrointestinal surgery, chronic pancreatitis, intestinal lymphoma, Crohn's disease, and Whipple's disease may present in a manner that makes these disorders indistinguishable from gluten-sensitive enteropathy.

Treatment

Exclusion of even traces of wheat gluten from the diet almost always results in restoration of small bowel structure and function to normal. This may take weeks or months, and until it is accomplished, dietary supplements and even parenteral administration of some essential nutrients are required. In initially refractory patients, corticosteroids may sometimes induce significant improvement.

Complications & Prognosis

Failure to recognize and treat the disease may result in severe malnutrition, the most important expression of which is irreversible stunting of growth. Delays in initiating treatment may result in a refractory state of malnutrition which does not respond to gluten exclusion and standard oral supplementation. Parenteral nutrition techniques may have to be used.

There is an increased risk of developing intestinal lymphoma (6–10% after a mean duration of the disease of 21 years). Malignancies of the foregut also occur in significantly higher incidence in subjects with celiac disease. A variable degree of splenic atrophy and immune deficiency may occur when the disease is of long duration. Otherwise it appears that rigorous dietary control can achieve a prognosis of normal health and longevity.

INFLAMMATORY BOWEL DISEASE

Major Immunologic Features

- Increased numbers of lymphocytes, plasma cells, and monocytes in the mucosa, and, in Crohn's disease, submucosa.
- Granulomatous response in intestinal lesions and regional lymph nodes in Crohn's disease.
- Presence of circulating antibodies to cytoplasmic lipopolysaccharide of colonic epithelial cells. Cross-reactive with that of *Escherichia coli* O14.
- Circulating lymphocytotoxic antibodies.
- Peripheral blood lymphocytes cytotoxic in vitro for isolated colonic epithelial cells.

General Considerations

The term "inflammatory bowel disease" describes 2 major categories of chronic bowel disease, which (though displaying some overlap) can usually be distinguished by clinical and histopathologic criteria. One is **Crohn's disease,** or **intestinal granulomatous disease,** which may affect any part of the gastrointestinal tract but occurs most frequently in the terminal ileum. The other is **nonspecific ulcerative colitis,** which is always confined to the rectum and colon.

In **Crohn's disease** the inflammatory lesion is primarily submucosal but ultimately involves the whole wall of the bowel from mucosa to serosa. Ulceration of the mucosa may extend to the serosa, causing local perforation and development of fistulas. Obstructive lymphedema and lymphadenoid hyperplasia occur. Noncaseating granulomas are found in 50–70% of the mucosal lesions and frequently in regional mesenteric lymph nodes.

The affected bowel becomes thickened and rigid and has been described as a "snake in rigor mortis." Narrowing of the lumen may result and cause obstruction. Two or more diseased segments may be separated by relatively normal bowel (**skip lesions**).

In **ulcerative colitis** the early inflammatory lesions are confined to the mucosa, which becomes diffusely inflamed. Mucosal blood capillaries dilate, and bleeding may occur on contact. A variable degree of ulceration is present. Submucosal inflammation is seen only in long-standing chronic cases. Infiltration of the lamina propria with mononuclear inflammatory cells is an invariable feature. Eosinophils are sometimes abundant. In acute attacks of varying severity, neutrophils abound. The **crypt abscess** is a focal collection of neutrophils in the deepest part of the lamina propria, contiguous with dilated crypts of Lieberkühn. Abscesses may rupture into the lumen of the gland. Ulcers may be superficial or involve the whole thickness of the mucosa, which in severe disease, may be lost and replaced by granulation tissue and simple epithelium. Granuloma formation is rare.

Immunologic Pathogenesis

The cause of inflammatory bowel disease is unknown. Specific infectious agents, both bacterial and viral, and nutritional, immunologic, and psychogenic factors have been postulated but without solid evidence. The disease (or diseases) displays a familial tendency, but no genetic pattern has emerged.

Much attention focused a decade ago on the induction of granulomatous lesions in the footpads and bowel wall of experimental animals by the injection of cell-free and ultrafiltered extracts of Crohn's lesions. Subsequently, it has been claimed that viruses of different morphologic characteristics can be cultured from Crohn's disease and ulcerative colitis lesions, but none of these findings has been confirmed. In any event, the granulomatous response, which appears to be a delayed hypersensitivity reaction, is nonspecific and may be induced by many inciting agents.

Although the finding of RNA antibodies with significant concordance in sera of some affected individuals and their spouses supports the infectious nature of inflammatory bowel disease, no epidemiologic evidence of infectivity has been obtained. Reports of remissions following exclusion from the diet of cow's milk or other proteins and of relapses following challenge with the same proteins are largely anecdotal, and data conflict about significant associations of inflammatory bowel disease with allergic conditions.

A. Humoral Factors:

1. Antibodies—No consistent differences in circulating immunoglobulins are found. Studies have revealed increased rates of turnover of IgG, IgM, C1q, and C3. In part, these reflect protein-losing enteropathy in the inflamed gut, but there also may be extravascular sequestration of the complement components.

Antibodies of IgG, IgA, and IgM classes against a colonic (but not small intestinal) epithelial lipopolysaccharide and against a similar antigen (derived from *E coli* O14) have been demonstrated in the blood of some patients with Crohn's disease or ulcerative colitis. These antibodies are found more frequently in affected children than adults. Their presence does not correlate with the extent, severity, or duration of disease, and they also occur in the blood of some apparently healthy relatives of patients.

2. Immune complexes—The presence of antigen-antibody complexes has been demonstrated by several techniques in the sera of patients with inflammatory bowel disease. Antibodies of IgG class are present in these complexes, but no antigens have been identified. Whether they are responsible for the extraintestinal complications of inflammatory bowel disease, some of which strongly resemble those seen in serum sickness, is speculative, as is the theory that they might serve to "arm" K cells, rendering them cytotoxic.

B. Cellular Immunity:

1. Skin tests—In inflammatory bowel disease, no consistent pattern of response to standard antigens such as PPD or DNCB has permitted the conclusion that anergy or hypersensitivity is a constant feature of either small or large bowel disease. Discrepant results, particularly in Crohn's disease of the small bowel, may be a consequence of nutrient deficiencies secondary to malabsorption or to quantitative differences in peripheral lymphocyte populations. In colonic inflammatory bowel disease, results of such tests have been similar to those in healthy controls. Reports of positive Kveim tests in patients with Crohn's disease suggest a link with sarcoidosis, as do successful attempts to transmit granulomatous lesions experimentally. Other reports are negative. The significance of granulomas remains unclear.

2. Tests of lymphocyte activation and MIF production, using circulating lymphocytes from patients with chronic inflammatory bowel disease, and PHA, PPD, colonic antigens, and *E coli* O14 antigen, have yielded variable results. Attempts to demonstrate significant disturbance of T cell to B cell ratios in the blood of healthy controls have been unsuccessful.

3. Circulating NK cells possessing surface Fc receptors for IgG from inflammatory bowel disease patients—and similar cells from the lamina propria of the colon of such patients—have been shown in some studies to be specifically cytotoxic in vitro against autologous colonic epithelial cells, but other studies have failed to confirm this. One recent study has emphasized the wide disparity in activity between blood and intestinally derived NK cells in inflammatory bowel disease, a disparity not seen in cells taken from healthy controls.

In summary, as a consequence of variations in the methods used for cell isolation and variable susceptibility of potential effector cells, clear evidence of colon epithelium-specific antibody-dependent or -independent cytotoxicity has yet to be obtained. An immune pathogenesis for inflammatory bowel disease is unproved.

Clinical Features

A. Symptoms, Signs, and Complications: Clinically, the major types of inflammatory bowel disease share certain features. Both are chronic diseases displaying unpredictable remissions and relapses. When Crohn's disease is confined to the colon, it may be clinically indistinguishable from ulcerative colitis. Symptoms range from mild to severe diarrhea. More severe attacks are accompanied by lower abdominal pain, fever, prostration due to dehydration, and passage of frequent and often bloody stools. Severity depends on the extent of colon involved. Crohn's disease tends to spare the rectum and may display skip lesions in the colon.

Complications occur in the region of the large bowel and anus. Free perforation is the most dangerous. It occurs in ulcerative colitis but is rare in Crohn's disease, in which local perforation and abscess formation are much more common. Fistula formation is rare in ulcerative colitis but common in Crohn's disease. The fistulas may extend to the perianal skin surface or other structures, such as the vagina and urinary bladder.

The gravest problem is the indisputable association of chronic inflammatory bowel disease of the colon with cancer of the colon, occurring 10 or more years after onset of the colitis. There is also evidence that cancer of the small bowel occurs more frequently when Crohn's disease is present than when it is not, but the rate is very low.

When Crohn's disease involves the small bowel, fever, abdominal colic, malabsorption of nutrients, and loss of weight occur. Frank intestinal bleeding is rare but occasionally may be massive. Occult blood is usually present in the stool. The lesion may present as a narrowed segment of small bowel, usually the terminal ileum, which may cause obstruction, or as a palpable, tender mass, most commonly in the right lower abdominal quadrant.

Extraintestinal Disease. In inflammatory bowel disease, lesions of the skin and oral mucosa, the joints, eyes, and liver and biliary tracts occur. The reason for these associations is not known. It has been suggested that immunologic factors, such as circulating immune complexes, which have been demonstrated in the sera of some patients by several techniques, may be responsible, but no association of extraintestinal lesions with such complexes has been demonstrated. Nor is there a high correlation of these lesions with severity of the bowel disease.

Skin and oral lesions are erythema nodosum, pyoderma gangrenosum, and aphthous ulceration. Ankylosing spondylitis occurs but almost exclusively in subjects with the HLA-B27 haplotype, the same association as that found in uncomplicated ankylosing spondylitis, whereas inflammatory bowel disease has not been associated with any HLA genotype. The episodic polysynovitis of inflammatory bowel disease, which tends to affect the larger joints, shows no such association. Rheumatoid factor is absent from the serum, and articular damage is mild or does not occur. Lesions of the eye are uveitis (including iritis) and episcleritis. The liver may show fatty infiltration, and there is variable inflammatory response in the portal tracts (portal triaditis), frequently closely related to the biliary ductules (pericholangitis). Radiologic techniques for visualizing the biliary tract have revealed a significant incidence of sclerosing cholangitis in inflammatory bowel disease, and there also appears to be an association with carcinoma of the biliary tract, which occurs at a younger age than in the general population.

B. Laboratory Findings: In acute attacks of inflammatory bowel disease, there is variable anemia due to acute blood loss. There may be precedent chronic blood loss and iron deficiency (microcytic, hypochromic) anemia. Evidence for megaloblastosis in Crohn's disease of the ileum is rarely present, although malabsorption of cobalamin occurs in a substantial number of such patients. Neutrophilia with shift to the left may be present, and eosinophilia is seen in some acute attacks. An elevated erythrocyte sedimentation rate is a valuable index of disease activity, especially in Crohn's disease. The stools contain no pathogens.

Immunologic Diagnosis

No tests have diagnostic value.

Differential Diagnosis

Lymphoma of the small intestine, Whipple's disease, giardiasis, tuberculous enteritis, infection with *Yersinia* or *Campylobacter*, gluten-sensitive enteropathy, Henoch-Schönlein purpura, and small bowel neoplasms are some of the conditions that may clinically resemble Crohn's disease of the small bowel.

Inflammatory bowel disease of the colon must be differentiated from *Shigella* and amebic dysentery and from so-called hemorrhagic colitis due to infection with *E coli* O157:H7 by the absence of specific pathogens in the stool and negative serologic findings for these organisms.

Treatment

Transfusions of fluids and blood are used to combat dehydration and hemorrhage, if necessary, combined with general supportive measures. Corticosteroids and sulfasalazine are used in various combinations for treating colitis. Azathioprine may be useful in allowing reduction of corticosteroid dosage. In Crohn's disease, metronidazole has been shown in trials to be useful. Recent reports of significant improvement of Crohn's disease of the small bowel following the use of oral elemental diets await confirmation.

Prognosis

A major characteristic of inflammatory bowel disease is unpredictability. Remissions follow relapses; relapses may appear to be induced by intercurrent infections, trauma, or emotional stress. Some patients may experience a single attack. In others, the disease of colon or small bowel pursues an unremitting course

leading to colectomy or surgical resection of the small bowel. In ulcerative colitis, total colectomy is followed by cure; in Crohn's disease, lesions may appear at any time after apparently successful removal of all affected tissue.

THE LIVER

Many of the immunologic phenomena associated with both acute and chronic liver disease are secondary to liver damage. Changes of diagnostic and possibly pathogenetic significance will be emphasized.

HEPATITIS A VIRUS (HAV) INFECTION

Major Immunologic Features

- Presence of IgM antibody to HAV is diagnostic of acute infection.
- Serum IgM increased.
- Presence of IgG anti-HAV alone indicates past infection and confers protective immunity.
- Chronic infection and chronic liver disease do not occur.

General Considerations

This viral infection, which is common in childhood, is enterally transmitted, with an incubation period of 4–6 weeks. The virus is an RNA (picorna) virus that replicates within the liver and probably small intestine, is excreted in the stools, and evokes a strong antibody response. The rapid rise in IgM antibody titer occurs at onset and is diagnostic of acute infection. IgG antibodies are present in high titer from the clinical onset and remain for life, conferring protective immunity.

The disease is often asymptomatic. In North America and Western Europe, by middle age, approximately 40% of people have immunity. The prevalence of infection increases by 10% per decade of life. The infection rate is greater in developing countries.

Fulminant hepatitis is rare, and the virus does not result in either chronic infection or chronic hepatitis. Periportal piecemeal necrosis, however, is seen more commonly than in type B and non-A, non-B hepatitis.

Immunologic Pathogenesis

During the acute phase, serum IgM concentration increases and large amounts of circulating immune complexes are present. The composition of these complexes is unknown. Low-titer smooth muscle antibody is often present. Low-titer IgM liver membrane antibodies (LMA) are found and may be causatively related to the piecemeal necrosis seen in these patients.

Prevention

Household contacts can be protected by administering immune serum globulin, 0.02 mL/kg intramuscularly, within 10 days of exposure.

HEPATITIS B VIRUS (HBV) INFECTION

Major Immunologic Features

- The virus is not cytopathic, and it is the host's immune response to the virus that causes liver damage.
- Infection may result in acute hepatitis, chronic active or persistent hepatitis, a carrier state with normal histologic features, or extrahepatic disorders including polyarteritis nodosa and membranoproliferative glomerulonephritis.
- Diagnosis is dependent on the presence of HBsAg or IgM anti-HBc, which differentiates acute from chronic infection.
- There are 2 phases of chronic infection: (1) the phase of active viral replication, when the patient is HBeAg-positive; and (2) the phase of HBsAg production in the absence of detectable viral replication, when the patient is anti-HBe-positive.
- Clearance of hepatocytes supporting HBV replication is probably mediated by cytolytic T cells sensitized to HBc/e antigens.
- Chronic infection occurs in 98% of neonates born to HBV carrier mothers. The active transfer of IgG anti-HBc from the mother to the infant, across the placenta, modulates the response to nucleocapsid proteins and may contribute to the development of persistent infection.
- Chronic infection occurring in 10% of adults may result from deficiencies of interferon production.
- Hepatocytes containing integrated HBV evade this elimination process and may undergo malignant transformation.
- Adenine arabinoside and alpha interferon may accelerate clearance of HBV. Homosexual patients respond less frequently.

General Considerations

Hepatitis B virus is parenterally transmitted, usually during therapeutic use of blood or blood products, sexual contact, or sharing of needles during drug abuse. The incubation period is 3–6 months. This infection may result in asymptomatic, symptomatic, or fulminant hepatitis, and in some cases, chronic infection with chronic hepatitis (Fig 25–3). More rarely, extrahepatic syndromes, including polyarteritis nodosa, membranoproliferative glomerulonephritis, polyneuropathy, papular acrodermatitis (Gionotti-Crosti syndrome), and essential mixed cryoglobulinemia may occur. Of those infected at birth, 98% develop chronic infection; and of those infected later in life, 10%.

The delta agent, an RNA virus that replicates only

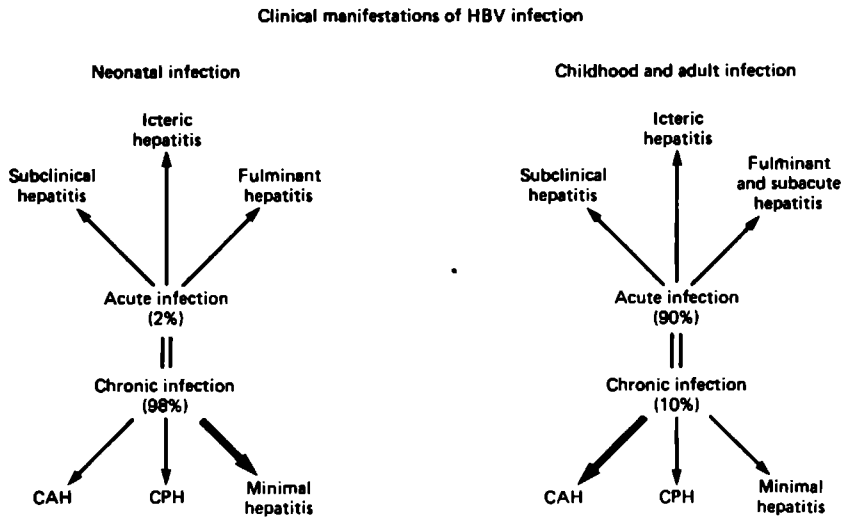

Figure 25–3. Clinical syndromes associated with acute and chronic HBV infection in neonatal, childhood, and adult life.

in patients with HBV infection, causes an acceleration of the course of the chronic hepatitis, so that patients progress to cirrhosis at an earlier age. The virus is probably cytopathic. This superinfection is, in the acute phase, diagnosed by the demonstration of delta agent or IgM anti-delta in the serum. In chronic delta agent infection, higher-titer IgG antibody is found.

Immunologic Pathogenesis

The virus itself is not directly cytopathic, and the diversity of lesions described in infected patients has been attributed to variation in the capacity of the host's immune response to eliminate or suppress the infective agent. In acute hepatitis, the mononuclear cell infiltrate in the liver is composed chiefly of cytotoxic T and NK cells. The lysis of infected hepatocytes, in association with production of virus-neutralizing antibody, is probably responsible for recovery. The humoral immune response during this acute phase has been studied in detail and is summarized in Fig 25–4. The appearance of anti-HBe and an HBV-reactive antibody is associated with the disappearance of HBeAg

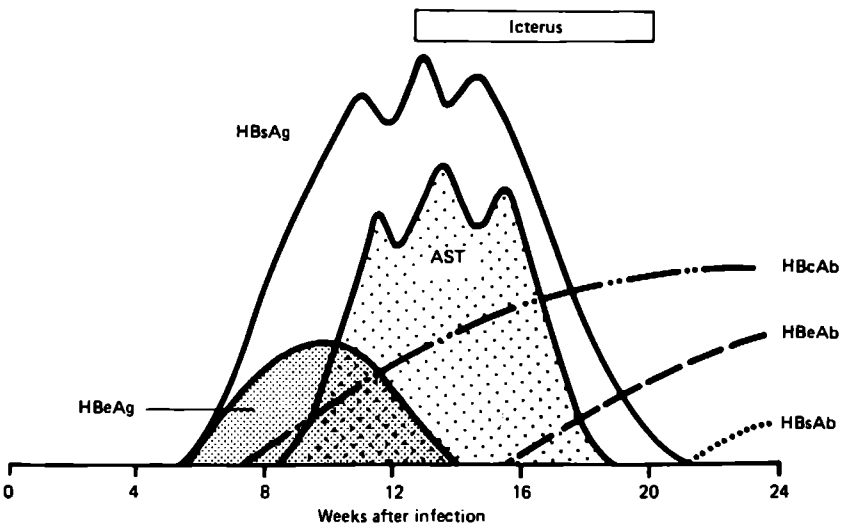

Figure 25–4. Acute type B hepatitis. Viral antigens and host immune response. The anti-HBc response is of the IgM class during the acute infection, and this is diagnostically useful. *Note:* During the phase of HBe antigenemia, HBV particles are present in blood. AST, aspartate aminotransferase (or GOT, glutamic oxaloacetic transaminase) is representative of liver cell necrosis.

and of HBV particles from the serum. The antibody conferring protective immunity is anti-HBs. This develops late in the illness, sometimes 1–2 months after disappearance of HBsAg. IgM anti-HBc is present from the first 2 weeks after onset of the infection and for 6 months thereafter. It is absent or in low titer during chronic infection. Cell-mediated immunity to the nucleocapsid proteins (HBc/e) displayed on the hepatocyte membrane is responsible for destruction of infected hepatocytes. This is modulated by the interferons.

A major enigma in this field is the mechanism by which the virus persists and why chronically infected subjects develop lesions varying from severe chronic active hepatitis to minimal hepatitis and extrahepatic syndromes. Defects in the mechanism of elimination of infected hepatocytes and in neutralization of infectious virus particles have been postulated to underly the chronic infection. Different defects have been postulated in neonatal and adult infections (Fig 25–5).

The presence in the serum of HBeAg, a low-molecular-weight component of the nucleocapsid of the virus, usually indicates the presence of virus particles in the circulation and therefore a state of relatively high infectivity. These patients readily transmit infection to sexual contacts, and inoculation of even small amounts of their blood during "needle-stick" injury will cause infection.

After several years of chronic infection, HBeAg disappears from the serum and HBeAb can be demonstrated (Fig 25–6). Although most patients have no virus particles visible by electron microscopy in their serum and are therefore of low or zero infectivity, when hybridization techniques are used to detect viral DNA, small numbers of virus particles can be detected in 20% of cases. Thus, HBeAg-positive patients and a small proportion of HBeAb-positive patients are infectious. Most HBeAb-positive patients are noninfectious. Clearance of HBV particles from the blood is usually marked by a period of lobular hepatitis and ele-

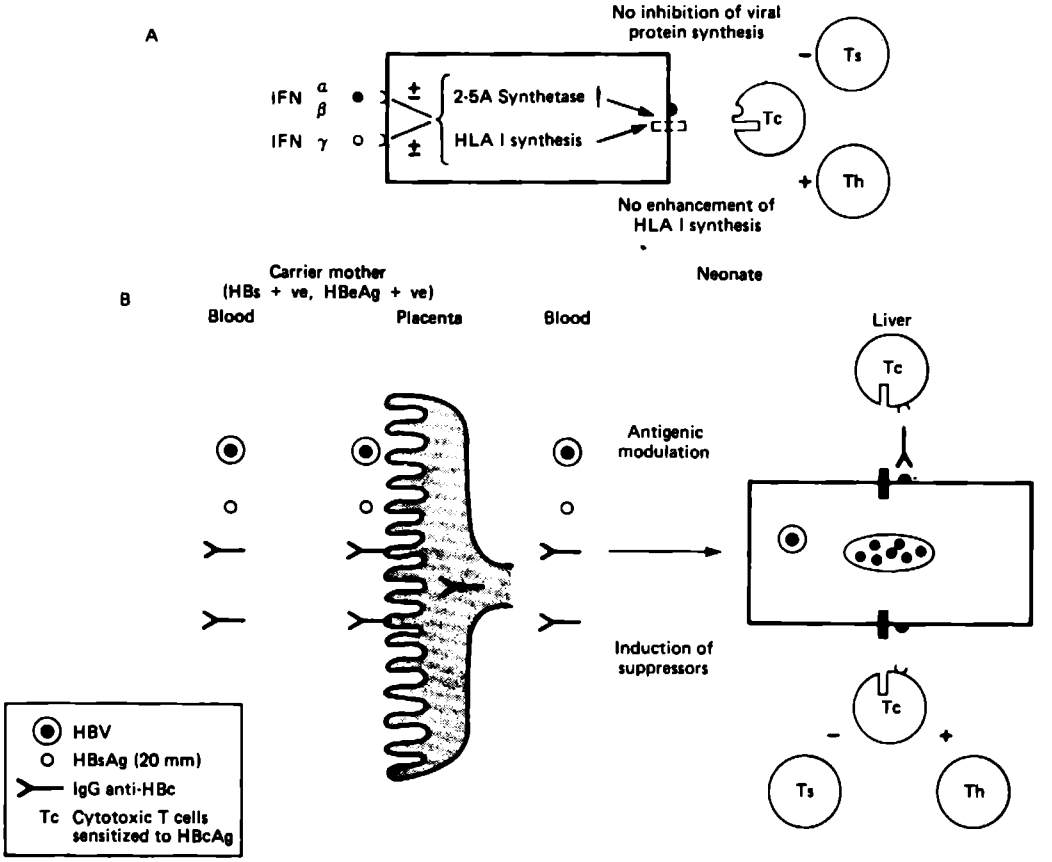

Figure 25–5. *A:* Postulated mechanism of carrier state arising in adult life. Relative deficiency of alpha interferon (IFN α) allows spread of the virus rapidly through the liver. The lack of IFN α results in failure of the cell-mediated immune response to destroy the infected liver cells. *B:* Postulated mechanism of carrier state arising in neonatal life. IgG anti-HBc passes from mother to infant by active placental transport. This antibody modulates the cell-mediated immune response to HBc antigen displayed on the hepatocyte membrane and allows the infected hepatocytes to persist.

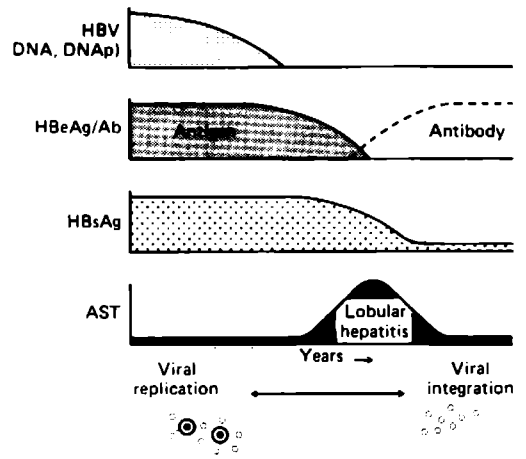

Figure 25–6. Virologic and serologic events during chronic HBV infection. During the phase of HBe antigenemia, HBV replicates in the liver and infectious particles (containing HBV-DNA and DNA polymerase [DNAp]) are found in the serum. Clearance of hepatocytes containing replicating HBV results in lobular hepatitis marked by a rise in aspartate aminotransferase (AST). After HBeAg to anti-HBe seroconversion, although virus particles disappear from the serum, HBs antigenemia continues and is now due to the secretion of noninfectious HBs antigen by clones of hepatocytes containing integrated HBV-DNA. Inflammatory activity in the liver is minimal at this phase of the infection.

vated transaminases. This transient exacerbation of the disease during conversion of HBe antigen to antibody represents immune lysis of hepatocytes supporting HBV replication. Cytolytic T cells probably sensitized to HBc/e antigen contribute to this elimination process.

The continued secretion of the viral coat protein (HBsAg), in the absence of active viral replication, probably represents a phase of infection in which the viral DNA has become integrated into the host DNA, so that the viral genome is transcribed and translated as if it were a part of the host. This integration event may explain why, late in the infection, patients develop primary liver cell cancer. How cells evade immune lysis is unknown. HBc/e antigen, the putative target for cytolytic T cells on the hepatocytes supporting HBV replication, is absent on these cells.

Low-titer antibodies to smooth muscle are present and are a reflection of the immune response to partially denatured antigens released from necrotic liver cells. Antibodies to single-stranded and double-stranded DNA are present.

Treatment of Chronic HBV Infection

Patients who are actively replicating the virus (HBeAg-positive) may be treated with either interferon or vidarabine (adenine arabinoside; Vira-A). Both of these agents will reproducibly inhibit viral replication, but in only one-third of cases is this long-lasting. In patients with prolonged responses, inhibition of viral replication is associated with falling HBsAg titers and ultimately with reduced hepatic inflammatory activity. It is anticipated that this therapy will be associated with an increased survival. Modified regimens, particularly those using the more water-soluble vidarabine monophosphate and lymphoblastoid interferon (Fig 25–7), hold greater promise and are currently under evaluation. Homosexual patients respond less frequently, probably because of secondary immunodeficiency. Immunostimulants have been tried to enhance the endogenous immune response, thus increasing the chance of clearance of the virus. Attempts at immunotherapy have been largely unsuccessful, but immunotherapy has the theoretic advantage of perhaps destroying clones of cells containing integrated HBV-DNA.

NON-A, NON-B HEPATITIS INFECTION

Major Immunologic Features

- Both parenteral (short [2–4 weeks] and long [6–12 weeks] incubation) and enteral (6–8 weeks incubation) transmission may occur.
- Chronic hepatitis occurs in 20–80% of cases of parenterally transmitted disease, but rarely or never after enteral non-A, non-B hepatitis.
- Diagnosis is dependent on exclusion of hepatitis A, hepatitis B, Epstein-Barr virus, and cytomegalovirus infection (by absence of IgM antibodies) and drug-induced hepatitis.
- Immunoglobulin concentrations are normal, and auto-antibodies (smooth muscle antibodies, antinuclear antibodies) are absent.

General Considerations

The non-A, non-B viruses are an important cause of sporadic (presumably enterally transmitted) and posttransfusion (parenterally transmitted) hepatitis. The diagnosis depends on the exclusion of hepatitis A and B virus infection by demonstrating the absence of IgM anti-HAV and HBsAg and IgM anti-HBc, respectively. Epstein-Barr virus (EBV) and cytomegalovirus infection must also be excluded by serologic tests. The acute illness is often mild, and many individuals are asymptomatic. The disease, however, has considerable importance in that 20–80% of parenterally infected individuals develop chronic infection and chronic hepatitis. This disease is also relatively benign in its course, but some patients do progress to cirrhosis. The importance of these viruses as etiologic factors in the causation of cryptogenic cirrhosis remains to be determined.

Serum transaminases fluctuate rapidly in the course of the chronic disease, and this pattern has been useful diagnostically.

The complexity of the problem has been increased by the knowledge that there are probably at least 3 non-A, non-B viruses. One, which is enterally trans-

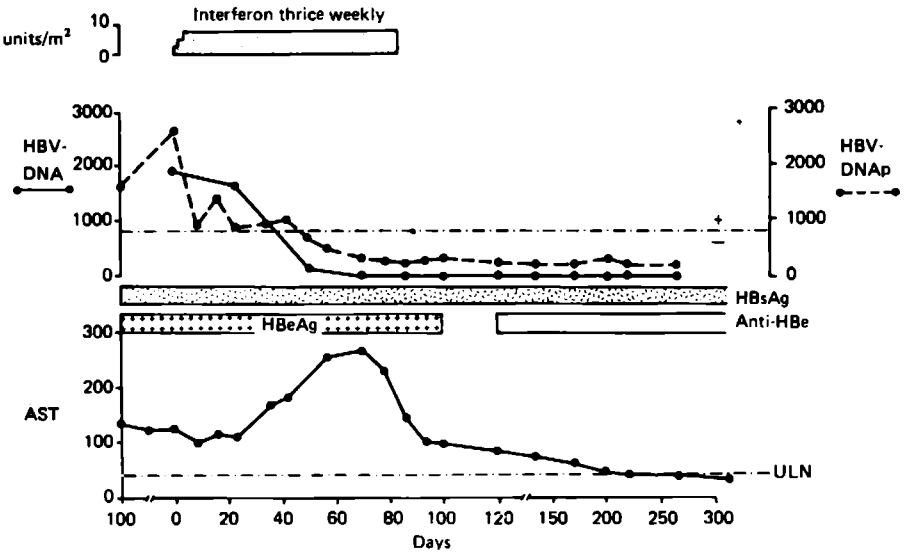

Figure 25–7. Response to lymphoblastoid interferon in chronic HBV infection. A 3-month period of interferon administration produced long-term inhibition of HBV replication and HBeAg to anti-HBe conversion. Note the rise in AST (aspartate aminotransferase), indicating lysis of hepatocytes during clearance of replicating HBV. The patient continues to produce HBs antigen owing to the presence of hepatocytes containing integrated HBV. This therapy reduces infectivity and inflammatory activity in the liver in a significant proportion of patients. (ULN = upper limit of normal.)

mitted and responsible for epidemic and possibly sporadic non-A, non-B hepatitis, does not result in chronic liver disease. There are, in addition, 2 parenterally transmitted viruses which can be characterized by the duration of their incubation periods (2–4 and 6–12 weeks) and which commonly cause chronic hepatitis.

Immunologic Pathogenesis

The parenterally transmitted viruses are probably cytopathic. Low-titer smooth muscle antibody occurs, but hyperglobulinemia is not evident until cirrhosis is present.

Serologic methods for the positive identification of this group of viruses are not yet clinically available.

Treatment & Prevention

There is no proved method of therapy. Immune serum globulin may confer protection if given before blood transfusion or with clotting factor concentrates. Its value in preventing infection in needlestick victims is unproved. The dosage and use are under investigation at this time, and therefore no definitive recommendation can be made.

AUTOIMMUNE CHRONIC ACTIVE HEPATITIS

Major Immunologic Features

- Autoantibodies to liver membrane, smooth muscle, and nuclear antigens.
- Genetic association with HLA-B8 and -Dw3.
- A defect in nonspecific immunoregulation associated with polyclonal hypergammaglobulinemia.

General Considerations

Chronic hepatitis is defined as chronic hepatic inflammation continuing without improvement for longer than 6 months. Inflammation of the intrahepatic biliary tree is usually excluded from this group of diseases. Several etiologic factors may initiate chronic hepatitis (Table 25–3). These include a primary defect in regulation of the immune response (autoimmune), persistent viral infection (type B and non-A, non-B hepatitis viruses), prolonged administration of drugs (oxyphenisatin, methyldopa, isoniazid, nitrofurantoin), alcohol, and Wilson's disease. In a substantial number of cases, no etiologic factor has been identified.

The distribution of the inflammatory infiltrate in the portal tracts and hepatic lobules allows a further classification (Fig 25–8) that is justifiable on prognos-

Table 25–3. Etiologic classification of chronic active hepatitis.

Autoimmune (lupoid)
Hepatitis B virus
Non-A, non-B virus (short incubation)
Non-A, non-B virus (long incubation)
Drug exposure (oxyphenisatin, methyldopa, isoniazid)
Alcohol exposure
Wilson's disease
Unknown cause

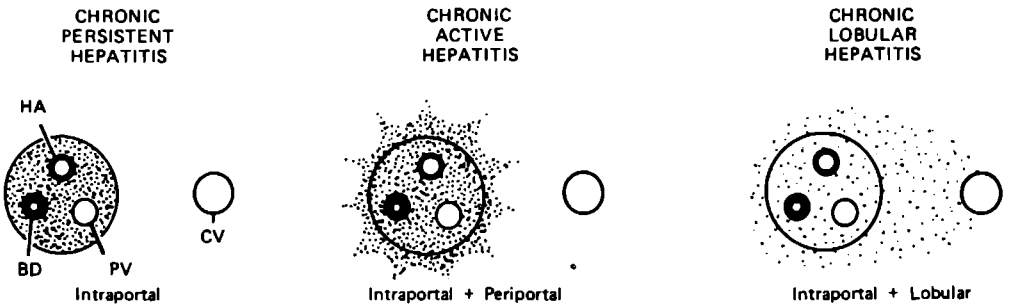

Figure 25–8. Anatomic classification of chronic hepatitis, dependent on the distribution of the inflammatory infiltrate within the hepatic lobule. HA = hepatic artery; BD = bile duct; PV = portal vein; CV = central vein. Clear circles represent limiting plate of portal tract. Black dots represent inflammatory cells.

tic grounds. These lesions may be seen with any of the etiologic factors.

In autoimmune chronic active hepatitis, there is mononuclear and plasma cell infiltration of the portal and periportal areas of the liver. Groups of mononuclear cells surround hepatocytes, some of which appear to be damaged, in a lesion called piecemeal necrosis. The lymphoid cells are predominantly T cells of the helper phenotype. Cytotoxic/suppressor cells are less frequent than in the virus-induced forms of chronic hepatitis. Three-quarters of the patients are female, and although all ages may be affected, peak incidences are seen between 10 and 25 years and 50 and 65 years. The disease has an insidious onset in most cases but occasionally presents abruptly with features suggestive of acute viral hepatitis. In these cases, acute symptoms that call attention to chronic hepatitis probably represent intercurrent (new) infections with type A, B, or non-A, non-B virus. Patients usually complain of general malaise for several months before jaundice is noted.

About half of patients have other immunologic disorders, including arthralgia or arthritis, vasculitis, ulcerative colitis, glomerulonephritis, fibrosing alveolitis, Hashimoto's thyroiditis, autoantibody-positive hemolytic anemia, leukopenia, thrombocytopenia, and diabetes mellitus.

The biochemical picture shows predominantly an elevation of aminotransferases with normal alkaline phosphatase. Hepatic synthetic function is reduced, manifesting as a low serum albumin and prolongation of the prothrombin time. There is often hematologic evidence of hypersplenism secondary to portal hypertension. Serum IgG is usually markedly elevated, with smaller changes in IgM and IgA levels. Antinuclear antibodies and antibodies to double-stranded DNA and to smooth muscle are present in high titer, usually greater than 1:40 by immunofluorescence. The term "lupoid chronic active hepatitis" was given to this syndrome because of the presence of antinuclear factor. More recently, additional autoantibodies have been described, including the liver-kidney microsomal and mitochondrial antibodies. Whether these antibodies define separate subgroups of differing origin remains to be determined.

Immunologic Pathogenesis

Both the number and function of suppressor cells are diminished in these patients, and this defect may be responsible for the development of autoantibodies reacting with liver membrane antigens. These antibodies are found in high titer in autoimmune chronic active hepatitis and are thought to mediate the lesion of periportal piecemeal necrosis. These antibodies are also found in patients with primary biliary cirrhosis who exhibit piecemeal necrosis. A reflection of the diminished activity of nonspecific suppressor cells is the high serum immunoglobulin concentration, particularly IgG. These patients also manifest autoimmune diseases affecting other organs, and it is suggested that the defect of the regulatory system is generalized. That this defect may be genetically determined is suggested by the finding of an increased incidence of autoantibodies and raised γ-globulin levels in the relatives of these patients and of a state of linkage disequilibrium of the disease with the human leukocyte antigens HLA-B8 and -Dw3. The latter association suggests that the inheritance of a gene or genes close to the B and D loci of the MHC on chromosome 6 predisposes to the development of the disease, either spontaneously or in response to some environmental trigger factor. Defects in antigen-specific suppressor cell function have also been described.

Treatment

Until we are able to correct the defect in the immunoregulatory system, immunosuppression represents the mainstay of therapy. Controlled trials have demonstrated that corticosteroids produce a prolongation of survival over a period of 10 years. Azathioprine may occasionally be added to allow maintenance with lower doses of steroids. The defect in immunoregulatory function returns to normal during steroid therapy. In most cases, relapse will occur on cessation of therapy. It seems probable that therapy must be continued for life.

PRIMARY BILIARY CIRRHOSIS

Major Immunologic Features

■ Ninety-nine percent of patients are mitochon-

drial antibody-positive.
- Diminished suppressor cell function.
- Increased serum concentrations of polymeric and monomeric IgM.
- Inability to convert from IgM to IgG antibody synthesis.
- Complement-activating serum factor—possibly immune complexes.
- Granulomatous infiltrate of intrahepatic biliary tree.

General Considerations

In this disease, a chronic granulomatous inflammatory process results in destruction of the intrahepatic biliary tree. These patients also exhibit lesions of the salivary, lacrimal, and pancreatic glands, scleroderma, rheumatoid arthritis, and thyroid disease. The finding of mitochondrial antibody in the serum of these patients led to the conclusion that the disease was probably of autoimmune origin.

The disease occurs commonly in middle-aged women, who present with symptoms of cholestasis. Liver function tests show an elevated alkaline phosphatase, often with normal transminases and bilirubin. The most prominent symptom is pruritus. The rate of progression of the disease is slow, and jaundice occurs late. Many patients remain anicteric for 10–20 years. Once jaundice develops, life expectancy is considerably reduced.

Immunologic Pathogenesis

Recently, evidence of an abnormality in the immunoregulatory system in these patients has been obtained. Both the concentrations and functions of suppressor T cells are markedly reduced. It is hypothesized that this abnormality allows the expansion of a clone of autoreactive lymphocytes with the potential to mount an immune response to both hepatic and nonhepatic ductular antigens. Similarity of the syndrome to chronic graft-versus-host disease, in which transplanted bone marrow cells attack the tissues of the body bearing a high density of HLA antigens, has led to the suggestion that the target antigen in primary biliary cirrhosis may be either a native or denatured HLA protein.

One result of the reduced suppressor cell activity is an increased rate of synthesis of IgM with failure of polymerization, leading to release of monomeric IgM. An additional abnormality of the humoral immune system is failure to convert from IgM to IgG antibody production. The protracted IgM response may be the result of failure of feedback inhibition because of the poor IgG antibody response.

Mitochondrial antibody is found in 99% of cases and is therefore a useful diagnostic test: The antibody binds to a nonenzymatic determinant on a lipoprotein component of the ATPase complex on the inner mitochondrial membrane. The role of this antibody in the pathogenic process is at present unclear.

These patients have large amounts of immune complex-like factors in their serum that result in activation of the classic and alternative pathways of complement. These complexes are responsible for some of the extrahepatic manifestations of the disease, including, in some cases, arthritis, arteritis, and glomerulonephritis. Their role in the genesis of hepatic granulomas is unknown.

Patients with primary biliary cirrhosis are severely anergic in delayed hypersensitivity skin tests. Serum factors appear to be important, and lately evidence has focused on the role of abnormal high- and low-density lipoproteins in the anergic state.

Treatment

Penicillamine, a copper-chelating and anti-inflammatory compound, has been shown to produce biochemical improvement, but whether it influences survival is controversial. Cyclosporine produces changes in the T cell subsets and a marked improvement in liver biochemistry. Clinical trials are continuing.

ALCOHOL- & DRUG-INDUCED HEPATITIS

Major Immunologic Features

- Direct toxic damage is followed by an immunologically mediated response to altered or denatured membrane and intracellular antigens (eg, alcohol and halothane hepatitis).
- Immune responses to altered antigens may be genetically determined (linkage of florid alcohol-induced hepatitis with HLA-B genes).
- Polyclonal hyperglobulinemia, particularly of IgA, in alcohol-induced liver disease.
- Depressed cell-mediated immune reations.

1. ALCOHOL-INDUCED LIVER DISEASE

Several factors contribute to the liver damage that occurs in patients consuming large amounts of alcohol. The alcohol or its metabolites are undoubtedly directly hepatotoxic, producing ultrastructural changes within a few hours after ingestion.

Since persons with similar exposure histories may respond with widely different degrees of liver damage, it is suspected that other factors than simply amount of alcohol consumed influence susceptibility. It seems likely that alcohol ingestion induces steatosis in all subjects, but progression to hepatitis and cirrhosis does not always occur. Some authors suggest that all patients with alcohol-induced cirrhosis have gone through a stage of hepatitis, but in other studies this march of event is less clear. The factors that determine the rate of development of the disease are not understood.

Recent studies have revealed an increased incidence of HLA-B8 in a group of patients with alcohol-induced hepatitis. The incidence in patients with steatosis is similar to that found in the normal popula-

tion, and subjects who have established cirrhosis without obvious hepatitis exhibit a low incidence of this phenotype. The authors suggest that in subjects who have progressed to cirrhosis with or without hepatitis, the mechanism of liver damage may be similar but that the presence of the gene or genes linked to HLA-B8 predisposes the subject to a more florid hepatitic component of the disease.

Immunologic Pathogenesis

A. Immune Response to Liver Cell Antigens: The liver biopsy specimen in alcohol-induced liver disease has some features that may be compatible with an immunologically mediated component of the disease process. Although a major feature is central necrosis and polymorphonuclear cell infiltration, in some cases the portal zones reveal a mononuclear cell infiltrate and stellate fibrosis. This periportal infiltrate is similar to that seen in chronic active hepatitis and may represent an immunologic reaction to hepatocytes at the point of entry of lymphocytes to the hepatic lobule. This possibility is supported by the observation that lymphocytes from patients with alcohol-induced hepatitis are sensitized to native and alcohol-altered liver cell antigens and are cytotoxic for alcohol-exposed hepatocytes.

The involvement of immune mechanisms in alcohol-induced liver disease is further supported by studies of the hepatic cellular infiltrate. Eighty percent of the cells in the liver of patients with alcoholic hepatitis are T lymphocytes and 20% are B lymphocytes. This contrasts with other forms of liver disease, in which the ratio of T to B lymphocytes is nearer 50:50. These findings are consistent with the suggestion that T lymphocytes have become sensitized to liver antigens and are mediating a tissue-damaging reaction.

The role of an immune response to Mallory's hyalin in the pathogenesis of alcohol-induced hepatitis is more difficult to determine. Hyalin is found in about 50% of cirrhotic patients with a history of alcohol abuse, but it is also found in Indian childhood cirrhosis, Wilson's disease, and primary biliary cirrhosis. The material is highly refractile, eosinophilic, cytoplasmic inclusion that probably represents a condensation of intracellular contractile filaments. Interest in this material has increased following the demonstration that patients with alcohol-induced hepatitis and cirrhosis are sensitized to the purified material. These patients also exhibit low-titer antibodies to smooth muscle and aggregated albumin, and it seems probable that all are a reflection of an immune response to intracellular proteins released from degenerating hepatocytes.

More recently, antibodies to alcohol-altered hepatocytes have been found in sera of patients with alcohol-induced hepatitis.

B. Altered Humoral Immunity: Although many of the immunologic features of alcohol-induced disease are common to other types of liver injury and presumably result from changes in hepatic phagocyte function, some features are peculiar to the disease. In alcohol-induced disease, one of the earliest changes is an increase in serum IgA concentration. This occurs at a stage when the liver either is normal or exhibits only a mild degree of steatosis. Since this class of antibody is produced in the intestinal wall, one explanation of this increase would be that alcohol exposure results in an increase in permeability of the mucosa, thereby allowing increased access of intestinal antigens to the IgA immunocytes of the lamina propria and mesenteric lymph nodes. This change may merely represent an epiphenomenon, but it also seems possible that the change in permeability allows absorption of factors that contribute to the induction of hepatic damage. Increased titers of antibody to *E coli* occur at this early stage, indicating that endotoxin is absorbed and may be one of the intestine-derived factors contributing to the ongoing liver damage.

C. Altered Cell-Mediated Immunity: Cell-mediated immunity is altered in patients with alcohol-induced liver disease. Since many of the changes are also found in other types of liver disease and in animal models of cirrhosis, it seems probable that they are the result of liver damage rather than the chemical effect of alcohol.

Eighty percent of patients with alcohol-induced liver disease fail to develop a delayed hypersensitivity reaction to a challenge with dinitrochlorobenzene and exhibit a significant decrease in responsiveness to streptokinase and to mumps, antigens to which the subjects are likely to have been already sensitized. This demonstrates a defect in the efferent part of the delayed hypersensitivity response but does not exclude a coexisting defect in the afferent limb. The normal response to croton oil rules out a defect in the inflammatory response. A normal in vitro response to T cell mitogens excludes an intrinsic T cell defect, but serum inhibitors of this response can be demonstrated.

Treatment & Prognosis

Withdrawal of alcohol is the main goal of therapy. In florid alcohol-induced hepatitis, corticosteroid therapy has been tried without significant beneficial effect. In poorly nourished alcoholics, some of the immunologic abnormalities may respond to improved diet.

The response to abstinence is variable and partly dependent on the degree of damage and consequent hepatic cirrhosis.

2. DRUG-INDUCED LIVER DISEASE

An increasing number of widely used and generally well-tolerated drugs can cause hepatic injury, ranging from a transient asymptomatic elevation of serum transaminases to clinically overt acute or chronic liver disease. These drug-induced states are often clinically, biochemically, and histologically indistinguishable from virally induced forms of liver injury, and this makes it difficult to establish a causal relationship between the drug and the disease. For this reason, the

list of drugs suspected of inducing liver injury is much longer than that of drugs which are of proved relationship.

Most drugs that injure the liver do so by one of 2 mechanisms. Some drugs or their metabolites are hepatotoxic by a chemical interaction with an essential structural component or metabolic enzyme system of the liver cell, whereas others involve a hypersensitivity reaction. In both cases, host factors may influence the probability of a significant adverse reaction. The rate of generation and detoxification of a toxic metabolite will influence both types of reaction, and immune response genes may be involved in determining whether or not a patient manifests an idiosyncratic hypersensitivity response.

Direct Hepatotoxins

Drugs toxic to liver usually cause acute hepatic necrosis. Prolonged administration causes protracted or repeated episodes of necrosis and may ultimately lead to the development of chronic liver disease. Salicylates and acetaminophen are 2 such drugs.

The onset of liver damage is immediate in all subjects exposed to sufficient dosage if direct toxicity is the mechanism. Such drugs also produce liver damage in animals, and are usually identified as hepatotoxic in preliminary animal toxicology studies. A causal relationship between the drug and the adverse reaction is readily established in an individual case by studying the effect of drug withdrawal on recovery.

Drugs Inducing Hypersensitivity Responses

A drug or its metabolite may induce liver damage by immunologic mechanisms. The drug may alter either the regulatory system of the immune response, so that reactions to self antigens are no longer suppressed; or it may alter hepatocyte antigens, so that they are no longer recognized as self components. In the former case, the ensuing disease may be multisystemic, whereas the alteration of liver antigens would be expected to produce an autoaggressive assault solely on the liver.

One can approach the problem from 2 directions. The first is to define the host factors that determine susceptibility; the second is to define the mechanism by which the drug produces liver damage.

The involvement of host factors is suggested by the observation that only a small minority of exposed subjects develop hepatic injury. This is in contrast to the high susceptibility rates to hepatotoxins (Table 25–4). The factors involved are poorly understood. The increased incidence in atopic subjects and the occurrence of identical reactions in several generations of a family suggest that hereditary factors exist. Genetic factors may influence the rate and form of metabolism of the drug, thereby influencing the rate of formation of immunogenic complexes of drug metabolite with cellular macromolecules. Some progress has been made by the demonstration that microsomal enzyme activity is genetically determined. In the field of immunology, evidence suggests that HLA phenotypes may be linked to immune response genotypes.

Table 25–4. Characteristics of toxic and hypersensitivity reactions.

	Direct Hepatotoxins	Hypersensitivity Reactions
Susceptibility	All subjects	Minority of subjects
Onset	Immediate	Delayed
Severity	Dose-related	Not dose-related
Animals affected?	Yes	No
Associated features	Other organs affected (renal damage)	Fever, arthralgia, rash, eosinophilia

The mechanism by which the drug initiates an autoaggressive immune response is also unknown. The drug may act as a hapten and combine with a membrane component of the hepatocyte, or it may denature a self antigen. This will result in a response to either the drug or a native or denatured liver cell antigen. Successful attempts to demonstrate these humoral and cellular responses are rare. When responses have been found, they are usually of the delayed hypersensitivity type. The paucity of positive data may in part be attributed to the insensitivity of the test systems but also probably stems from failure to test with both the drug and its metabolites complexed to the appropriate carrier molecule.

Although it is theoretically possible that the autoimmune reaction may continue after removal of the offending drug, this does not usually occur. Thus, for chronic liver disease to develop, prolonged exposure over several months would be necessary. Establishing a causal relationship between the drug and the liver lesion is a major problem. Withdrawal of the suspected drug usually results in clinical and biochemical improvement, and in a clinical setting, this is all that can be done. Rechallenge may be permissible in mild reactions, but in more severe cases it carries the risk of a severe exacerbation. Very little help can be derived from biochemical, histologic, or serologic studies. For example, chronic active hepatitis induced by methyldopa, oxyphenisatin, or isoniazid is indistinguishable on biochemical and histologic grounds from other forms of the disease, and in many cases the autoimmune markers (antinuclear antibody, LE cells) are also present.

The type of liver injury may be classified on histologic and biochemical grounds as hepatocellular or cholestatic and, in some cases, as a mixture of the 2. There are no specific features that incriminate one drug rather than another. The history usually points to exposure to a specific drug.

Hepatitic Reactions

This type of reaction has been reported with monoamine oxidase inhibitors, oxyphenisatin, methyldopa, halothane, aminosalicylic acid (PAS), and sulfonamides (not discussed here). If exposure is prolonged, the patient may develop chronic liver disease.

A. Monoamine Oxidase Inhibitors: Mono-

amine oxidase inhibitors produce a predominantly hepatitislike picture. Iproniazid was the first recorded example, and this was followed by phenelzine, pheniprazine, and isocarboxazid—all hydrazine derivatives. The reaction may be severe, and fulminant cases have been reported.

Isoniazid is a member of this group used mainly in tuberculosis chemotherapy. Ten percent of patients show increased transaminase levels during the first 2 months of therapy, and liver biopsy shows a mild hepatitis. Only a minority of patients (less than 1%) develop symptomatic liver disease, and fatalities are rare. The reaction is usually mild, and the transaminases return to normal when the drug is stopped. It is possible, however, that continued administration may occasionally induce chronic active hepatitis.

B. Oxyphenisatin: A constituent of many laxatives, oxyphenisatin has been associated with hepatocellular damage. Only a minority of exposed persons react adversely, usually after at least 6 months of continual use. Most patients develop an acute hepatitic illness, but some present with chronic active hepatitis indistinguishable from the lupoid variety. The LE test and antinuclear factor are often positive, and hyperglobulinemia develops. The illness subsides when the drug is stopped, and challenge leads rapidly to worsening liver function as disclosed by appropriate tests.

C. Methyldopa: This drug produces mild subclinical abnormalities in transaminases in 5% of recipients. The frequency of this reaction and its occurrence early after ingestion suggest a direct toxic mechanism. In a minority of patients, a more severe hepatic reaction occurs 3–16 weeks after starting treatment. The prodromal symptoms are similar to those of acute viral hepatitis, and the patient becomes jaundiced. The Coombs test and tests for antinuclear factor and smooth muscle antibody may be positive. The patient usually recovers uneventfully when the drug is stopped, but occasionally the course if fulminant or a stage of subacute hepatic necrosis proceeds to chronic active hepatitis and then cirrhosis.

D. Halothane: Halothane is now established in controlled trials as a cause of postoperative jaundice. Many of the features of the hepatitis strongly suggest a hypersensitivity mechanism. The reaction occurs 8–13 days after the first operation, and earlier after subsequent exposures. Pyrexia usually precedes the development of jaundice by 2–3 days and may be accompanied by eosinophilia. The outcome is good in the majority of cases. However, if the patient becomes icteric, the mortality rate is very high—up to 20%.

Early reports of mitochondrial antibodies in these patients' sera have not been confirmed, but a recent study did demonstrate that 40% of cases were positive for liver-kidney microsomal antibody. Demonstrations of cell-mediated immunity to the drug by lymphocyte transformation or leukocyte migration inhibition are also conflicting. Sensitization was demonstrated by some authors but not by others.

The presence of antibodies and T lymphocytes sensitized to halothane-altered hepatocyte antigens has recently been described in patients with halothane hepatitis but not in those undergoing halothane anesthesia without complication.

E. Aminosalicylate (PAS): PAS reactions involving the liver are common and usually part of a generalized reaction. Pyrexia, rashes, and arthralgias accompany the hepatitis. Cholestatic features are common.

Cholestatic Reactions

This type of reaction occurs in association with phenothiazines, oral hypoglycemics (chlorpropamide), and antithyroid drugs (thiouracil). Only chlorpromazine will be discussed here.

Chlorpromazine-induced reactions are often of a mixed hepatitic/cholestatic type. The reaction occurs 1–3 weeks after starting treatment in approximately 0.5% of patients receiving the drug. The reaction is unrelated to the dose and may occur several weeks after stopping the drug. If chlorpromazine is given a second time, approximately 40% suffer a relapse; it is postulated that the remaining subjects, who do not respond to challenge, are desensitized by subsequent doses.

Prodromal symptoms of fever and rash and blood and tissue eosinophilia all support a hypersensitivity mechanism as the cause of the syndrome. Liver biopsy shows cholestasis and a marked portal mononuclear and eosinophilic infiltrate. There is variable hepatitis. The prognosis is good. There are 2 reports of progression to biliary cirrhosis.

Granulomatous Reactions

Granulomatous reactions are seen in patients treated with phenylbutazone and sulfonamides. Although clinical and histologic features suggest an immunologic basis for the lesion, serologic tests and tests for lymphocyte sensitization have been unrewarding.

INFLUENCE OF LIVER DISEASE ON THE IMMUNE RESPONSE

Hyperglobulinemia and depressed cell-mediated immunity are common to most forms of chronic liver disease, and it seems probable that these changes are a result of liver damage. This is supported by the observation that similar changes can be induced in rats when they are rendered cirrhotic.

Phagocytic Function

Phagocytic function is markedly altered in patients with hepatic cirrhosis. This results in changes in antigen distribution, which is a major factor in determining the characteristics of the ensuing humoral and cellular immune responses.

Antigens enter the circulation from the gastrointestinal tract, the larger ones via the mesenteric lymphatics and the smaller ones via the mesenteric venous circulation. The liver, which is an important phagocytic organ, may therefore receive intestinally derived antigen either directly, via the portal circulation when it

acts as a filter interposed in series with the rest of the body, or it may receive antigen from the systemic circulation via its arterial supply and in this situation acts as a filter in parallel. The hepatic phagocytes render antigens nonimmunogenic, while splenic and lymph node-derived macrophages serve to enhance immunogenicity. The distribution of antigen between liver and spleen (and other lymphoid organs) will therefore influence the magnitude of the immune response. Diversion of antigen from the liver to the spleen will enhance the immune response. Changes in the phagocytic function of the liver have an effect on both portal and systemic routes of immunization.

Immune complexes are cleared from the portal and systemic blood by the hepatic sinusoidal phagocytes. Large complexes that fix complement are avidly cleared by the liver, whereas smaller complexes, which do not fix complement, are cleared by the spleen. In hepatic cirrhosis, these functions are impaired, and complexes accumulate in the plasma. In most cases these complexes do not result in significant activation of C3 and do not cause tissue damage. The composition of such complexes is unknown, but it seems probable that many will contain food and bacterial antigens derived from the gut.

Endotoxins are phagocytized and detoxified by the liver: they can be demonstrated in portal blood but not in systemic blood in normal subjects. Endotoxinemia has been demonstrated during fulminant hepatic failure and in some subjects with established cirrhosis, presumably as a result of impaired hepatic clearance of this substance. It is suggested that it plays a significant role in the renal malfunction that often accompanies these diseases.

Humoral Immunity

The altered handling of antigen in subjects with hepatic cirrhosis has a significant effect on the humoral immune response. This is seen most clearly in the response to putative thymus-independent antigens, which are not influenced by changes in cell-mediated immunity that accompany the development of chronic liver disease. It seems probable that the increased *E coli* titers found in patients with alcohol-induced cirrhosis, chronic active liver disease, and primary biliary cirrhosis are the result of this phenomenon.

The response to thymus-dependent antigens is more complex, involving the cooperation not only of macrophages and B lymphocytes but also of T lymphocytes. When patients with alcohol-induced cirrhosis, chronic active liver disease, and primary biliary cirrhosis were immunized intravenously with the bacteriophage ØX174, a thymus-dependent antigen, the primary and secondary responses were significantly decreased when compared with the responses of normal subjects. In the presence of increased responses to thymus-independent antigens, this implies that the cooperating functions of T cells are reduced, so that B cells challenged with thymus-dependent antigens cannot respond to the increased antigenic stimulus.

In addition to the quantitative changes in the humoral response of subjects with hepatic cirrhosis, there are also qualitative changes. In normal subjects, during a secondary response, more than 90% of the antibody is IgG, whereas in patients with primary biliary cirrhosis, chronic active hepatitis, and alcohol-induced cirrhosis, the percentage of IgM antibody is much increased. This relative failure to change from IgM to IgG antibody production during the evolution of the immune response is also compatible with a defect in helper T cell function.

The relationship of the increased viral antibody titers, which have been described in chronic liver disease, to altered mononuclear phagocytic function is more vexed. Small increases in titer to lipoprotein-coated viruses such as herpes simplex, cytomegalovirus, influenza A, rubella, and measles are seen in HBsAg-positive and HBsAg-negative chronic active hepatitis, alcohol-induced cirrhosis, and primary biliary cirrhosis, but the 6-fold increase in titer to measles and rubella viruses seen in lupoid chronic active liver disease is peculiar to this disease. It seems probable that the one- to 2-fold increase in titer to several viruses, which is common to all types of chronic liver disease, is a reflection of the altered immune function which occurs in any type of chronic liver injury but that the specific association of a high-titer response to measles and rubella with lupoid chronic active liver disease is an indication of the presence of an additional diathesis in the immune system of these patients.

The cumulative effect of these increased humoral responses is readily seen in the hypergammaglobulinemia that accompanies any form of experimental or natural chronic liver disease. The fact that this change is probably secondary to alterations in the mononuclear phagocytic system of the liver should not be allowed to direct attention away from the increased IgM of primary biliary cirrhosis and IgA of alcohol-related disease—changes specific to these diseases that may therefore give further clues to their pathogenesis.

Cell-Mediated Immunity

The incidence of positive delayed hypersensitivity skin tests to common bacterial and viral antigens is decreased in alcohol-related liver disease, chronic active hepatitis, and primary biliary cirrhosis. This occurrence in all types of liver disease suggests that this is in part secondary to the chronic liver disease.

The delayed hypersensitivity response involves the cooperation of macrophages and T cells in the presence of various serum factors that may enhance or inhibit the response. The afferent limb of the system, whereby cells become sensitized to the antigen concerned, has not been evaluated in patients with chronic liver disease because of the limitations of the test systems.

The efferent limb requires that T lymphocytes be present in adequate concentrations; that they be sensitized to and recognize the antigen under test; and that they be capable of producing the lymphokine mediators of the delayed hypersensitivity reaction. The con-

centration of peripheral blood T lymphocytes measured by rosetting techniques is diminished in HBsAg-positive and HBsAg-negative chronic active liver disease, alcohol-related disease, and primary biliary cirrhosis, and concentrations of null cells are increased. The demonstration that some null cells can be converted into E-rosetting mature T lymphocytes suggests that some null cells are either immature T cells or T cells which are altered because of the biochemical changes associated with chronic hepatocellular and, to a lesser extent, cholestatic liver disease. The presence of plasma or serum inhibitors of T lymphocyte function may also contribute to the anergy seen in patients with chronic liver disease. Serum factors that inhibit mitogen transformation of lymphocytes have been demonstrated in primary biliary cirrhosis, chronic active hepatitis, and alcohol-induced liver disease, as well as in an animal model of hepatic cirrhosis. Increased macrophage suppressor cell activity, which is dependent on the level of antigen stimulation of the spleen, has also been described in animals and patients with hepatic cirrhosis. Although the humoral and cellular inhibitors of T lymphocyte function are readily demonstrated in vitro, their functional importance in vivo remains uncertain. Many of the inhibitors appear to be common to several types of liver disease and are therefore probably a reflection of the altered metabolic state in these conditions.

Immunologically Active Plasma Proteins

The liver is the major site of synthesis for many plasma proteins, some of which have either a regulator or effector role in the immune response.

A. Alpha-Fetoprotein (AFP): Alpha-fetoprotein is produced by the entodermal cells of the foregut, particularly the liver. It is present in high concentration in the plasma of the fetus and mother. Within a few hours after birth, the concentration starts to fall, and by 1 year, adult levels are attained (10–20 ng/mL).

Increased concentrations of AFP have been demonstrated in 90–95% of primary hepatocellular carcinomas. Smaller increases (< 500 ng/mL) are seen following acute hepatic necrosis, acute viral hepatitis, and in patients with chronic liver disease, particularly those with macronodular cirrhosis. In these circumstances, the increase is believed to be a reflection of an increased rate of liver cell division (ie, regeneration). Thus, this protein is synthesized at an increased rate during hyperplastic and neoplastic growth.

The immunosuppressant properties of AFP were shown to be dependent on the induction of a suppressor cell. The presence of sialic acid residues on the protein appears to be essential for these biologic effects. More recently, other workers have failed to confirm these observations.

B. Alpha Globulins: These are a complex group of proteins that are produced by the liver and have immunoregulatory properties. Pregnancy-associated globulin inhibits T cell functions. It is increased mainly in pregnancy but also in patients with chronic liver disease and in cancer. Alpha$_2$ macroglobulin is an important inhibitor of both the complement and coagulation systems. It has recently been suggested that it has immunoregulatory properties in relation to K cell function. Increased concentrations are found in primary biliary cirrhosis and also HBsAg-negative chronic active hepatitis.

C. Complement Components: Complement components are produced by either the mononuclear phagocytes or hepatocytes and are often reduced in acute and chronic liver disease.

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Pulmonary Diseases

26

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The primary function of the lung is to facilitate exchange of gases between ambient air and blood. There are 2 important consequences of this gas exchange function: (1) the lung is exposed to a wide variety of airborne environmental antigens, and (2) the lung must serve as a conduit and filter for the entire circulating blood volume. The lung is therefore continually exposed to a variety of air- and blood-borne agents that have the potential to trigger inflammation, infections, or immune processes.

Our understanding of the pathogenesis and natural history of various immunologic lung diseases has been aided by various animal models and, in humans, by bronchoalveolar lavage. Prior to the development of this technique, information regarding human disease was available only through histologic examination of lung tissue or studies of peripheral blood. Bronchoalveolar lavage allows repeated sampling of the inflammatory and immune effector cells at sites of disease within the lung. The cells present in bronchoalveolar lavage are similar to those identified with histologic studies of lung tissue.

GOODPASTURE'S SYNDROME

Major Immunologic Features

- Circulating anti-glomerular basement membrane (anti-GBM) antibodies.
- Linear deposition of immunoglobulin and complement in basement membranes of renal glomeruli and pulmonary alveoli.

General Considerations

Goodpasture's syndrome is a disease of unknown cause occurring predominantly in young men and characterized by the triad of pulmonary hemorrhage, glomerulonephritis, and antibody to basement membrane antigens. The history may include an antecedent viral infection or inhalation of volatile hydrocarbons. Intrapulmonary hemorrhage may be insignificant or may be severe and life-threatening; if prolonged, iron deficiency anemia may result. Renal involvement is often rapidly progressive, with oliguric renal failure occurring within weeks to months after clinical onset.

Immunologic Pathogenesis

In over 90% of cases, circulating IgG antibody can be demonstrated early in the course of the disease. These antibodies are directed against renal tubular, renal glomerular, and pulmonary alveolar basement membranes, where they can be demonstrated in characteristic linear deposition by indirect immunofluorescence; they are often accompanied by C3 deposition. Antibody bound to basement membrane probably activates the complement cascade, resulting in the generation of chemotactic factors for various inflammatory cells. The inflammatory cells release reactive oxygen species and proteolytic enzymes that destroy the renal tubular, renal glomerular, and pulmonary alveolar membranes. Recent studies demonstrate that the antigenic site is probably within type IV collagen, present in basement membrane throughout the body but perhaps more vulnerable within the lung and kidney owing to their unique filtration functions and blood flow characteristics. Anti-GBM antibody eluted from diseased kidneys will react with normal GBM but not normal lung tissue; antibody eluted from diseased lung tissue has been shown to bind to GBM of normal kidney. The level of circulating anti-GBM antibody shows no consistent correlation with severity or prognosis of the disease.

Clinical Features

Pulmonary manifestations include pulmonary hemorrhage with or without hemoptysis and shortness of breath. The chest x-ray may reveal hilar infiltrates, which may fluctuate in intensity depending upon the degree of intra-alveolar hemorrhage. Bronchoalveolar lavage demonstrates hemosiderin-laden macrophages with or without erythrocytes. Gross or microscopic hematuria, proteinuria, and increased blood urea nitrogen and serum creatinine with a decreased creatinine clearance are characteristic features of renal involvement.

Immunologic Diagnosis

The presence of circulating antibody to glomerular basement membrane and the demonstration of linear deposits of antibody, with or without C3, along the basement membrane of glomeruli or alveolar septa are diagnostic.

Differential Diagnosis

Pulmonary hemorrhage with renal failure may be seen in Wegener's granulomatosis, systemic lupus erythematosus, polyarteritis nodosa, legionnaires' disease, congestive heart failure, and renal vein thrombosis with pulmonary embolism. These disorders lack the constellation of clinical, pathologic, and

immunologic features on which the diagnosis of Goodpasture's syndrome is based.

Treatment & Prognosis

Since the disorder may be rapidly fatal, it is imperative that it be diagnosed and treated rapidly. For patients who are acutely ill, initial membrane plasma exchange to remove the anti-GBM antibodies and corticosteroids plus cytotoxic agents are utilized. This type of therapy frequently halts progression of the disease and maintains renal function if instituted early enough in its course, ie, before interstitial fibrosis and irreversible glomerular damage have occurred. Bilateral nephrectomy, followed by renal transplantation after circulating anti-GBM antibodies have disappeared, has also been utilized as therapy for this disease. Recurrences, however, have been reported following renal transplantation.

HYPERSENSITIVITY PNEUMONITIS

Major Immunologic Features

- Caused by sensitization to specific inhaled environmental antigens.
- Serum precipitating antibodies present (principally IgG) to specific environmental antigens.
- Early (4- to 6-hour) response to inhaled antigens probably mediated by formation of immune complexes.
- Chronic exposure to antigen may result in a granulomatous interstitial lung disease.

General Considerations

Hypersensitivity pneumonitis (extrinsic allergic alveolitis) is an immunologically induced disorder of the alveolar walls and terminal airways of the lung that develops in response to repeated inhalation of a variety of organic dusts and other agents by a susceptible host. Although many agents that can cause hypersensitivity pneumonitis have been identified (Table 26–1), most are unusual causes of the disease, and a few well-documented syndromes are associated with most cases of clinical hypersensitivity pneumonitis. Most cases involve exposure to the same agents, especially the thermophilic actinomycetes. These organisms are encountered upon exposure to "moldy" hay, grain, silage, pet birds, humidification, and cooling or heating systems. Hypersensitivity pneumonitis may also be caused by simple chemicals, such as isocyanates. The diagnosis of hypersensitivity pneumonitis is based upon a number of clinical, radiographic, physiologic, pathologic, and immunologic criteria none of which alone are pathognomonic. The most important aspect of treatment is avoidance of the inciting agent.

Table 26–1. Examples of hypersensitivity pneumonitis.

Antigen	Source of Antigen
Thermophilic actinomycetes*	Contaminated hay, grain, silage
Parakeet, pigeon, dove, and chicken serum proteins	Avian droppings
Thermophilic actinomycetes, *Aureobasidium pullulans*, ameba, other	Contaminated water in humidifiers, aerosols, vaporizers, sprays
Wood dust; *Alternaria*	Oak, cedar, mahogany dusts; pine and spruce pulp
A pullulans, other	Contaminated sauna steam
Thermophilic actinomycetes	Contaminated bagasse (sugar cane)
Thermophilic actinomycetes, other	Mushroom compost
Pullularia, Graphium species	Redwood sawdust
Cryptostroma corticale	Maple bark
Coffee bean dust	Coffee beans
Infested wheat flour	*Sitophilus granarius* (wheat weevil)
Toluene diisocyanate (TDI)	Porcelain surfacing catalyst
Toluene diisocyanate (TDI), methylene diisocyanate (MDI), phthalic anhydride, vinyl chloride, other	Polyurethane foam and insulation, synthetic rubber manufacturing, meat wrapping and labeling, other

*Thermophilic actinomycetes include *Micropolyspora faeni, Thermoactinomyces vulgaris, T saccharii, T viridis,* and *T candidus.*

Immunologic Pathogenesis

Evidence exists for both an immune complex-mediated reaction and a delayed hypersensitivity reaction in the lungs of patients with hypersensitivity pneumonitis. The early (acute) reaction is characterized by the presence of increased numbers of polymorphonuclear leukocytes in alveoli and small airways and probably occurs in response to the formation of immune complexes in the lung. The formation of immune complexes after inhalation of specific antigen is not surprising, since these patients have preexisting precipitating antibodies to the antigen in serum and locally in the lung. In some patients, this early lesion evolves into an inflammatory process characterized primarily by mononuclear cells and the formation of granulomas. This latter delayed hypersensitivity reaction is thought to occur in response to repeated exposure to antigen and adjuvantlike materials. Consistent with these pathologic findings, bronchoalveolar lavage of patients with hypersensitivity pneumonitis shows an increase in the numbers of both macrophages and T lymphocytes. The percentages of T lymphocytes are usually markedly increased. In patients with recent or continued exposure to the inciting agent, the numbers of neutrophils and eosinophils may also be increased. The T lymphocytes present in lavage fluid are primarily suppressor/cytotoxic cells that express CD8 surface antigens. In patients with very recent exposure to antigen, however, the numbers of helper/inducer T cells that express CD4 antigens may increase in lavage fluid. These latter observations suggest that the delayed hypersensitivity component of this disorder is modulated by regulatory T cells.

Clinical Features

The clinical features of hypersensitivity pneumoni-

tis vary depending upon the nature of the individual's exposure and sensitivity to the offending antigen. Hence, the relatively clearly defined features of acute, subacute, and chronic patterns of exposure exist as extreme examples of what should be considered as a continuum of signs and symptoms.

Acute hypersensitivity pneumonitis is usually caused by intermittent exposure to antigen over a relatively short period of time and is characterized, symptomatically, by a nonproductive cough, dyspnea, fever, chills, malaise, and myalgia that typically begin 4–6 hours after exposure and clear within 18–48 hours after exposure ends. The patient is asymptomatic between attacks, and in the absence of other disease, the physical examination is unremarkable. Laboratory features of the acute episode include leukocytosis with a leftward shift, an elevated erythrocyte sedimentation rate, and elevated immunoglobulins. Total IgE is usually normal.

Subacute hypersensitivity pneumonitis often appears insidiously over a period of weeks, marked by cough and dyspnea. The disorder may progress and the patient may appear acutely ill, with tachypnea, tachycardia, fever, bibasilar respiratory rales, and, in severe cases, marked dyspnea and cyanosis; wheezing is notably absent. Radiographs reveal soft reticular or patchy interstitial infiltrates with or without multiple small, poorly defined nodules in a bilateral pattern that may spare the bases and apices. However, the x-ray picture may also appear normal. Pulmonary function testing shows a restrictive defect with decreased compliance and, in severe cases, hypoxemia and a decreased diffusing capacity.

Prolonged exposure to antigen may result in **chronic hypersensitivity pneumonitis;** symptoms include gradually progressive exertional dyspnea and cough. Many patients have no history consistent with acute or subacute disease. The chest x-ray shows changes of interstitial fibrosis with prominent involvement of the peripheral lung fields; honeycombing and cardiomegaly are seen in the end stages. Pulmonary function testing most commonly shows a restrictive defect, although an obstructive pattern may be seen.

Immunologic Diagnosis

Evaluation of the patient for the presence of serum precipitins against suspected antigens is an important part of the diagnostic workup. The diagnosis of hypersensitivity pneumonitis is not established solely by the presence of these antibodies, since precipitins merely indicate sufficient exposure to an antigen to generate an immunologic response. In this regard, precipitins are frequently found in individuals exposed to appropriate antigens who demonstrate no other evidence of hypersensitivity pneumonitis. False-negative results may occur because of poor quality of antigens used for the tests or an inappropriate choice of antigens. The lack of standardized nonirritating antigens and of proved controlled studies makes skin testing and inhalational challenge useful only for experimental purposes. Similarly, in vitro tests of cell-mediated immunity do not correlate consistently with clinical hypersensitivity pneumonitis and cannot be recommended in routine diagnostic workups.

Differential Diagnosis

The diagnosis of hypersensitivity pneumonitis should be considered in any patient with a history of recurrent "pneumonias" or with interstitial lung disease. Pulmonary mycotoxicosis (or "atypical" farmer's lung) closely resembles hypersensitivity pneumonitis and occurs in patients massively exposed to moldy silage. It is manifested by fever, chills, and cough within a few hours of exposure. Precipitins are not present, suggesting that this disease occurs in individuals not sensitized to the inhaled antigen.

Chronic hypersensitivity pneumonitis may be difficult to distinguish from a number of other interstitial lung disorders such as idiopathic pulmonary fibrosis, interstitial lung disease associated with a collagen vascular disorder, and drug-induced lung diseases. No history of use of appropriate drugs and no evidence of a systemic disorder usually exclude the presence of drug-induced lung disease or a collagen vascular disorder. Lung biopsy may be required to differentiate chronic hypersensitivity pneumonitis from idiopathic pulmonary fibrosis.

The lung disease associated with acute or subacute hypersensitivity pneumonitis may resemble other disorders that present with systemic symptoms and recurrent pulmonary infiltrates. These disorders include the collagen vascular disorders, drug-induced lung disease, allergic bronchopulmonary aspergillosis, and other eosinophilic pneumonias. Eosinophilic pneumonia is often associated with asthma and is typified by peripheral eosinophilia, neither of which is a typical feature of hypersensitivity pneumonitis. Allergic bronchopulmonary aspergillosis is sometimes confused with hypersensitivity pneumonitis because of the presence of precipitating antibodies to *Aspergillus fumigatus*.

Treatment & Prognosis

The mainstay of effective therapy is early diagnosis and avoidance of the offending antigen. In some cases, modification of the environment (eg, use of air-filtering units, removal of humidifier) or the use of tightly fitting face masks will suffice. If exposure is unavoidable and the patient remains symptomatic, a change in occupation or environment must be recommended. In the acute and subacute stages of disease, the prognosis is excellent, since the disease is usually totally reversible. Corticosteroids may hasten resolution and decrease the severity of acute symptoms. Although chronic hypersensitivity pneumonitis will usually show varying degrees of irreversibility despite avoidance of antigen exposure, these patients frequently improve without therapy following environmental control. In many patients, however, a trial of prednisone may be useful to obtain maximum reversibility of the disease. Most patients will not benefit from long-term corticosteroid therapy if there is no

further exposure to antigen. The prognosis of chronic hypersensitivity pneumonitis is dependent upon the severity of the irreversible changes.

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

Major Immunologic Features

- Atopic individuals with history of asthma.
- Immediate cutaneous hypersensitivity to *Aspergillus* antigens.
- Evidence of central bronchiectasis.
- Eosinophilia of sputum and blood.
- Elevated total serum IgE and specific anti-*Aspergillus* IgE.
- Serum IgG precipitins to *Aspergillus fumigatus*.

General Considerations

Allergic bronchopulmonary aspergillosis (ABPA) ocurs in patients with atopic asthma whose respiratory tracts become colonized with *Aspergillus*. Intrapulmonary growth results in high concentrations of antigenic material within the lung. It is unclear what predisposes some asthmatics to this disease. If untreated, ABPA also results in a characteristic proximal bronchiectasis and fibrotic lung injury. Early treatment with corticosteroids can control the disease and prevent progression to irreversible bronchiectasis.

Immunologic Pathogenesis

It is likely that the disorder is initiated with colonization of the respiratory tract in atopic hosts by *Aspergillus* species. The bronchial asthma of ABPA, in part, involves an IgE-mediated degranulation of mast cells, resulting in increased airway resistance. The bronchiectasis associated with this disorder is thought to result from the generation of immune complexes in the proximal airways. These immune responses can be elicited in affected individuals by antigen challenge. Intradermal antigen injection results in an immediate wheal-and-flare reaction. In over half of patients, the acute skin test response to antigen is followed by an Arthus reaction with edema and erythema. This reaction usually begins at 3 hours, with peak reactivity at 8 hours and resolution within 24 hours. Immunofluorescent studies of skin biopsies reveal deposition of IgG, IgM, IgA, and complement. Antigen inhalation initially results in acute bronchoconstriction. The late pulmonary reaction begins about 10 hours after antigen inhalation and may last 1–3 days. It is frequently associated with constitutional symptoms, including fever, malaise, and anorexia. A late reaction consisting of increased airway resistance with wheezing may also occur. This late reaction responds poorly to bronchodilators but is blocked by pretreatment with corticosteroids. It is believed that an equivalent of the cutaneous Arthus reaction accounts for the characteristic late pulmonary reaction to antigen, and, if it is recurrent, it may result in irreversible proximal bronchiectasis. In some patients, there is also evidence that a portion of the airway disease is caused by a delayed hypersensitivity reaction. Lung biopsy specimens in these patients show granulomas and mononuclear cell infiltrates in bronchial and peribronchial tissue.

Clinical Features

Patients are typically young to middle-aged adults with a history of recurrent asthma. Nonspecific complaints include anorexia, weight loss, progressive fatigue and general "flulike" symptoms. A history of sputum production is common; this may be characterized by expectoration of brown sputum plugs containing high concentrations of eosinophils and fungal elements. Hemoptysis and chest pain are common complaints. Physical findings include generalized wheezing and localized rales. The chest x-ray may demonstrate recurrent, migratory infiltrates or evidence of central bronchiectasis and mucus plug impaction. Patients with chronic disease may also demonstrate evidence of pulmonary fibrosis on chest x-rays. Bronchography shows a characteristic proximal bronchiectasis that spares the periphery. Pulmonary function testing shows an obstructive defect; in a small percentage of patients with chronic disease, a restrictive pattern may also be seen.

Immunologic Diagnosis

There is no single pathognomonic clue to diagnose ABPA; rather, its diagnosis rests on a constellation of findings in a susceptible host with an appropriate clinical history (Table 26–2). Primary diagnostic criteria include a history of asthma and pulmonary infiltrates, the findings of peripheral blood eosinophilia, elevated serum IgE, precipitating antibodies against *Aspergillus* antigens, and immediate cutaneous hypersensitivity to *Aspergillus* antigens. The absence of the latter finding casts doubt upon the diagnosis of ABPA. Patients with ABPA will exhibit most of these findings; however, pulmonary infiltrates may not occur or may not be documented. Corticosteroid therapy for the chronic asthma may mask the eosinophilia, elevated IgE, and pulmonary infiltrates. Precipitating antibodies may require special techniques for their demonstration.

Table 26–2. Diagnostic features of allergic bronchopulmonary aspergillosis (ABPA).

Main diagnostic criteria
Bronchial asthma
Pulmonary infiltrates
Peripheral eosinophilia (> 1000/μL)
Immediate wheal-and-flare response to *Aspergillus fumigatus*
Serum precipitins to *A fumigatus*
Elevated serum IgE
Central bronchiectasis
Other diagnostic features
History of brownish plugs in sputum
Culture of *A fumigatus* from sputum
Elevated IgE (and IgG) class antibodies specific for *A fumigatus*

Differential Diagnosis

ABPA should be differentiated from extrinsic asthma and other chronic airway diseases such as cystic fibrosis that may also be associated with colonization of the airways with *Aspergillus* species, hypersensitivity pneumonitis, parasitic infections and drug reactions associated with pulmonary infiltrates and blood eosinophilia, Löffler's syndrome, chronic eosinophilic pneumonia, allergic angiitis and granulomatosis of Churg and Strauss, and the hypereosinophilic syndrome.

Treatment & Prognosis

The main therapeutic agents in ABPA are bronchodilators and corticosteroids. The bronchodilators usually improve the asthmatic component of the disease. Corticosteroids improve the asthmatic component and are also required to prevent irreversible bronchiectasis. Prednisone must be given in moderate to high doses over a prolonged period, usually several months, until pulmonary infiltrates have cleared and IgE levels have decreased. A few patients may then discontinue prednisone without further evidence of disease. Most patients, however, will require continued corticosteroid therapy, although often at low doses. No efficacy has been found for the use of antifungal agents or hyposensitization.

The prognosis is related to the degree of irreversible bronchiectasis or fibrotic lung disease. When the disease is recognized early, before irreversible changes have developed, most patients do well and usually require only small doses of prednisone. The prognosis for patients who develop fixed airway disease or fibrotic lung disease is more guarded; these patients may develop progressive lung disease and die of end-stage lung disease despite therapy.

IDIOPATHIC PULMONARY FIBROSIS

Major Immunologic Features

- Immune complexes in peripheral blood and lung.
- Increased intra-alveolar IgG with or without increased serum IgG.
- Presence of nonspecific "autoimmune" antibodies.
- Evidence of restrictive (fibrotic) lung disease.
- Absence of systemic disease.

General Considerations

Idiopathic pulmonary fibrosis is an interstitial lung disease of unknown cause. The diagnosis is one of exclusion; ie, the patient must not have a history of exposure to agents known to cause interstitial lung disease or an underlying disease that is associated with the development of interstitial lung disease. Some patients show improvement after treatment with corticosteroid or cytotoxic therapy, and survival for 10–15 years following diagnosis has been documented; however, most cases end in death within 5 years.

Immunologic Pathogenesis

The agents that trigger the development of this disorder are still unknown. It is known, however, that early active disease is associated with the presence of immune complexes in serum and in lung. Although these immune complexes may trigger an inflammatory process in the lung via activation of the complement cascade, there is no evidence that this process occurs in the lungs of these patients. It has been shown, however, that immune complexes present in the lung stimulate alveolar macrophages to release various factors that appear to play an important role in pathogenesis. One factor released by alveolar macrophages is a lipid chemotactic factor that attracts neutrophils and eosinophils from blood into the lung. Although not specifically identified, this factor is probably leukotriene B_4, the major lipid chemoattractant released by alveolar macrophages. Alveolar macrophages in this disease also release a variety of growth factors for fibroblasts that increase the numbers of lung fibroblasts and the deposition of collagen, and thus eventually lead to pulmonary fibrosis. These observations are consistent with the pathologic features of the disease, ie, increased numbers of polymorphonuclear leukocytes and generalized fibrosis of the lung parenchyma.

Clinical Features

Patients commonly are first seen during the fifth to seventh decades of life with a history of gradually progressive exertional dyspnea and fatigability. If they have compensated by reduction in activity, the disease may be far advanced at the time of presentation. A superimposed illness, especially a viral or flulike episode, is often associated with initiation of symptoms. Physical examination reveals dry bibasilar rales ("Velcro rales"), and there usually is clubbing of the digits. In advanced cases, cyanosis and evidence of cor pulmonale may be present. The chest x-ray shows interstitial fibrosis with basilar predominance; honeycombing of the lungs occurs in later stages. Pulmonary function testing shows a restrictive defect with decreases in lung volumes and diffusing capacity. Arterial blood gases reveal normal or slightly decreased P_{O_2}, which drops markedly with exertion. P_{CO_2} is usually decreased unless the patient has severe end-stage disease. The blood count and differential white count are normal, and the erythrocyte sedimentation rate may or may not be elevated.

Immunologic Diagnosis

Since this is a diagnosis of exclusion, there are no specific confirmatory tests. Serologic abnormalities may include a positive test for antinuclear antibodies or rheumatoid factor, the presence of increased amounts of immunoglobulins, or immune complexes. Various immunologic tests are utilized primarily to exclude the presence of other interstitial lung disorders. The pathologic findings are also nonspecific and reveal a generalized inflammation and fibrosis of the alveolar capillary membrane and small airways of the

lung. The primary use of the lung biopsy is also to exclude other disorders. Bronchoalveolar lavage is characterized by increased numbers of both alveolar macrophages and polymorphonuclear leukocytes. The most characteristic feature is an increased percentage of both neutrophils and eosinophils. Although these lavage findings may be useful to determine the activity of the lung disease and, thus, the need for therapy, they are not specific for this disorder and are seen in a variety of other interstitial lung disorders.

Differential Diagnosis

The differential diagnosis includes a long list of interstitial lung diseases of both known and unknown cause. The principal considerations, however, include sarcoidosis, hypersensitivity pneumonitis, interstitial lung disease associated with collagen vascular diseases, and interstitial lung disease associated with certain inorganic dust exposures.

Treatment & Prognosis

Therapy is directed at suppressing active inflammation (alveolitis) and thus preventing further loss of function. High doses of corticosteroids may result in improvement and stabilization of pulmonary function in some patients but must be continued over a long period, usually indefinitely. Cytotoxic agents have also been reported to benefit some patients. Supplemental oxygen, particularly during periods of exercise, allows a more active lifestyle. Appropriate care must also be given to prevent and to promptly treat any pulmonary infection, since these are especially severe in these patients. Although some patients may improve with corticosteroid or cytotoxic therapy, these agents do not prevent progression of the disease and death.

SARCOIDOSIS

Major Immunologic Features

- Circulating T lymphocytopenia.
- Anergy to various skin test antigens.
- Polyclonal hypergammaglobulinemia.
- Circulating immune complexes in early disease.
- Systemic granulomatous disease.
- Intense cellular immune response localized to sites of disease.

General Considerations

Sarcoidosis is a multisystem granulomatous disease of unknown cause. It is worldwide in distribution and most commonly presents during young adult life with cutaneous, ocular, or pulmonary manifestations, although a significant number of patients are detected while asymptomatic on the basis of an abnormal chest x-ray. More than 90% of patients have pulmonary manifestations; about one-fourth of these will develop permanent loss of pulmonary function. In the USA, the disease is much more prevalent and more severe in the black than in the white population.

Immunopathogenesis

The agent that triggers the development of the disorder we recognize as sarcoidosis is unknown. Nevertheless, it is known that the earliest lesion is an accumulation of inflammatory cells in involved tissues. These inflammatory cells are primarily monocytes/macrophages and T cells, although an increased number of B cells and plasma cells are also present. This initial lesion sets the stage for the formation of granulomas by these inflammatory cells.

The central core of the granulomas is made up of a number of activated mononuclear phagocytes, including epithelioid cells, multinucleated giant cells, and macrophages. These cells are all derived from blood monocytes. Activated T cells found at the periphery of granulomas play an important role in the pathogenesis of sarcoidosis by releasing a variety of lymphokines. Monocyte chemotactic factor attracts monocytes, the building blocks of the central core of the granulomas, to sites of disease. The T cells also release a variety of mediators, such as macrophage migration inhibitory factor (MIF) and gamma interferon, which are necessary for the activation of macrophages, a characteristic feature of this disorder. Interleukin-2, which is released by T cells, maintains the increased numbers of T cells at sites of disease by 2 mechanisms: (1) by acting as a specific chemotactic factor that attracts T cells from blood to sites of granuloma formation, and (2) by stimulating T cells at sites of granuloma formation to proliferate.

The process of granuloma formation appears to be modulated by immunoregulatory T cells. In this regard, helper T cells usually augment various immune processes, while suppressor T cells usually dampen these same processes. In patients with active disease, the numbers of T cells in involved tissues are markedly increased and are primarily helper T cells. In contrast, fewer T cells are present in involved tissues in patients with inactive disease and are primarily suppressor T cells.

Polyclonal hypergammaglobulinemia, which consists of increased levels of antibodies to a variety of agents, including viruses and mycobacteria, is in part related to granuloma formation. T cells at sites of granuloma formation release a variety of mediators that nonspecifically activate surrounding B cells to differentiate into immunoglobulin secreting cells.

Clinical Features

Acute sarcoidosis may present with fever, erythema nodosum, iritis, and polyarthritis, and this constellation of findings strongly suggests the disorder. Most often, however, patients relate an insidious onset of fatigue, weight loss, malaise, weakness, anorexia, fever, sweats, nonproductive cough, and progressive exertional dyspnea. Patients also may be asymptomatic, and the disorder is often suggested by findings noted on a routine chest x-ray. Chest x-ray has in fact been the most important means of detecting the disease, demonstrating one of 4 types of involvement: type O, no roentgenographic abnormalities;

type I, bilateral hilar adenopathy alone; type II, hilar adenopathy and parenchymal abnormalities; and type III, parenchymal abnormalities without hilar adenopathy. These radiographic abnormalities are correlated to some extent with prognosis.

Pulmonary function studies may be normal or may reveal evidence of restrictive lung disease characterized by reduced lung volumes, decreased diffusion capacity, and exercise-induced hypoxemia. Patients with advanced disease may also exhibit evidence of an obstructive ventilatory defect.

Although the lung is the most commonly involved tissue, sarcoidosis can affect any organ of the body. All patients should be evaluated for the presence of granulomatous uveitis and heart disease because of the risk of blindness and death from cardiac arrhythmias.

Table 26–3. Diagnostic features of sarcoidosis.

Main diagnostic criteria
Compatible clinical picture
Histologic evidence of a systemic granulomatous disease compatible with sarcoidosis
No evidence of exposure to an agent known to cause granulomatous disease
Other diagnostic features
Circulating T lymphocytopenia
Anergy to various skin test antigens
Polyclonal hypergammaglobulinemia
Circulating immune complexes
Increased numbers of macrophages and helper T cells in bronchoalveolar lavage fluid
Positive Kveim test
Increased serum angiotensin-converting enzyme activity

Immunologic Diagnosis

The diagnosis of sarcoidosis can be firmly established only by the following criteria (Table 26–3): (1) a compatible clinical picture, (2) histologic evidence of a systemic granulomatous disease compatible with sarcoidosis, and (3) no evidence of exposure to an agent known to cause granulomatous disease. The disorder is also frequently associated with peripheral blood T lymphocytopenia, anergy to various skin tests, hypergammaglobulinemia, circulating immune complexes, increased serum angiotensin-converting enzyme activity, and increased numbers of macrophages and helper T cells in bronchoalveolar lavage fluid. These latter findings suggest but are not specific for sarcoidosis. The Kveim reaction, a cutaneous test associated with sarcoidosis, is largely of historical interest owing to unavailability of antigen and availability of other diagnostic tests.

Differential Diagnosis

Sarcoidosis must be differentiated from a variety of granulomatous diseases, including various infectious diseases, hypersensitivity pneumonitis, berylliosis, drug reactions, and certain malignant neoplastic diseases. Type III sarcoidosis must also be distinguished from a variety of other interstitial lung disorders.

Treatment & Prognosis

Progressive loss of lung function, cardiac disease, granulomatous uveitis, and central nervous system disease are absolute indications for corticosteroid therapy. Therapy should be continued as long as the disease is active. The least toxic regimens, such as alternate-day therapy, should be utilized when possible. Corticosteroids may also be used to relieve many of the symptoms associated with sarcoidosis such as joint involvement and erythema nodosum. In general, the prognosis of sarcoidosis is good if clinically significant central nervous system, cardiac, and pulmonary involvement are not present. About 5–10% of patients eventually die of the disorder.

PULMONARY VASCULAR LEUKOSTASIS

This pulmonary disorder became clinically apparent after the widespread use of hemodialysis. A profound, transient neutropenia was noted to occur shortly after dialysis treatment was started. Subsequent studies demonstrated that this is related to exposure of patients' plasma to new dialyzer membranes and was caused by a complement-mediated aggregation of neutrophils and monocytes in the lung. Membrane exposure of serum activates the alternative complement pathway, generating C3a and C5a. The generation of C5a has recently been shown to cause increased expression of a granulocyte surface glycoprotein associated with increased aggregability. The reaction does not occur in granulocytopenic patients nor in animal studies when plasma is pretreated to inactivate complement.

Pulmonary functional changes associated with this phenomenon include a decrease in P_{O_2} commencing within 30 minutes of initiation of dialysis and lasting up to 6 hours; concurrently, a decrease in diffusing capacity and an increase in alveolar-arterial oxygen gradient occurs. This phenomenon is usually of no significance in otherwise normal dialysis patients but may produce morbidity in patients with significant cardiac or pulmonary disease. Furthermore, a similar phenomenon has been demonstrated during nylon-fiber leukopheresis and cardiopulmonary bypass and may also occur in adult respiratory distress syndrome caused by circulating endotoxin.

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Cardiac Diseases

27

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POSTPERICARDIOTOMY SYNDROME (Postcommissurotomy Syndrome, Postmyocardial Infarction Syndrome, Dressler's Syndrome)

Major Immunologic Features

- Rise in antibodies to viral agents.
- Circulating antibodies to cardiac tissue.
- Circulating lymphocytes sensitized to mitochondrial extracts of cardiac tissue.

General Considerations

The postpericardiotomy syndrome is a febrile illness that occurs in 25–30% of patients who have had cardiac surgery or in patients with nonpenetrating trauma of the chest and in 1% of patients following myocardial infarction. The illness is characterized by persistent fever that appears 1–2 weeks following cardiac or pericardial injury. Fever is often associated with chest pain and pericardial and pleural effusions. The pathogenesis of the postpericardiotomy syndrome is not known. It is seen less frequently following cardiac surgery in infants under 2 years of age or in adults over age 70. It is more common in patients who have had extensive cardiac surgery, and particularly in patients who undergo heart valve surgery. A high frequency of elevated antibody titers to certain viruses is encountered in patients with this syndrome. The appearance of circulating antibodies that bind to cardiac muscle and of lymphocytes that are sensitized to cardiac muscle mitochondrial extracts suggests that an immunologic process initiated by surgery or a viral infection may be responsible for this syndrome.

Immunologic Pathogenesis

Circulating antibodies against cardiac antigens can frequently be demonstrated following traumatic, surgical, or vascular injury to cardiac or pericardial tissue. Hemagglutination, immunofluorescence, and complement fixation assays have detected these antibodies in up to 80–90% of patients who have had cardiac surgery. The immunofluorescence studies demonstrated 3 main patterns of staining of cardiac muscle fibers: (1) diffuse sarcoplasmic, (2) sarcolemmal-subsarcolemmal, and (3) intermyofibrillar. The sarcolemmal-subsarcolemmal pattern of staining was present in both cardiac and skeletal muscle but not in muscle in other organs.

Patients with postpericardiotomy syndrome have shown 2 patterns of antiheart antibody response. Some patients show a marked rise in circulating antibodies around the seventh to tenth postoperative days, with persistence of the antibody for about 1–2 months. Other patients experience a lower antibody response during the first or second postoperative week, and the antibody declines and disappears earlier than in the previous group. Of note is that patients without antiheart antibody following cardiac surgery do not develop the postpericardiotomy syndrome. This contrasts with the post-myocardial infarction illness described by Dressler in which many patients develop anticardiac antibodies without demonstrating any symptoms. De Scheerder and coworkers found that the presence of antiheart antibody correlated with the severity of the postpericardiotomy syndrome. Circulating antiheart antibody was present in the serum of all patients with postpericardiotomy syndrome and in only 5% of serum samples from controls who had undergone noncardiac surgery.

Total hemolytic complement levels and C3 and C4 inactivation products were studied in a group of patients several weeks after myocardial infarction. Although serum complement levels normally rise slightly after myocardial infarction, in a few cases there is evidence for activation of complement via the classic pathway. Some of these patients develop symptoms suggestive of postpericardiotomy syndrome, and although the evidence is limited, this suggests that postpericardiotomy syndrome (and similar clinical disorders) might result from circulating immune complexes of cardiac antigen and anticardiac antibody.

Cell-mediated immunity was studied recently in postsurgical and post-myocardial infarct patients with the postpericardiotomy syndrome. Migration inhibition of peripheral blood leukocytes was determined in the presence of 3 different antigenic preparations from normal human hearts. These preparations consisted of (1) whole saline extracts of heart tissue, (2) mitochondria, and (3) myoglobin isolated from heart tissue. Two-thirds of the patients who had undergone cardiac surgery or had a myocardial infarct showed significant migration inhibition of their peripheral blood leukocytes in the presence of the mitochondrial antigen but not the other 2 antigen preparations. The level of inhibition usually peaked during the second postoperative week, declined to normal during the fourth week, and

then increased again during the fifth to sixth weeks in those with clinical evidence of the postpericardiotomy syndrome.

The role of viral infection was also investigated. A 4-fold or greater rise in circulating antibody to one of several viruses tested (adenovirus, coxsackievirus B1–6, cytomegalovirus) occurred in 70% of patients with clinical postpericardiotomy syndrome and positive antiheart antibodies. Antibody rises to cytomegalovirus and coxsackievirus B4 were the most frequently encountered, but the frequency of rises to any one virus varied from year to year. These findings suggest that the rise in antibody reflected infection with the virus or viruses most prevalent in the community at the time of the surgery.

The above observations suggest that an immunologic stimulus resulting in the formation of antibodies to cardiac tissue antigens and the sensitization of lymphocytes to cardiac tissue mitochondria occurs in one-third of patients following cardiac surgery or myocardial infarction. This process may be initiated by release of cardiac antigens into the circulation either through the trauma of tissue manipulation during surgery or following infection of heart tissue by a certain virus during or after surgery. The prompt response of patients to immunosuppressive agents suggests that an immune stimulus, humoral or cellular, may be responsible for the clinical symptoms associated with the postpericardiotomy syndrome.

Clinical Features

A. Symptoms and Signs: The illness is characterized by the appearance of fever after the first week following surgery or myocardial infarction. The fever may be intermittent or persistent for several weeks. Pericarditis is associated with the presence of precordial chest pain, sometimes referred to the shoulder, and by the presence of a friction rub. Pleural involvement is manifested by pain on deep inspiration.

B. Laboratory Findings: The laboratory findings are nonspecific and are usually of little help in diagnosis of the syndrome. There may be leukocytosis, an increased red cell sedimentation rate, and a positive test for C-reactive protein.

The ECG may show changes of pericarditis as well as findings associated with the underlying cardiac disease, such as myocardial infarction, which preceded the onset of the syndrome. Radiographic studies may show cardiomegaly and pleural effusion. Echocardiography should confirm the presence of pericardial fluid.

Immunologic Diagnosis

A positive test for circulating antiheart antibodies, if available, will help confirm the diagnosis.

Differential Diagnosis

Postpericardiotomy syndrome should be differentiated from the postperfusion syndrome, in which hepatosplenomegaly is a distinguishing finding, and from other postoperative illnesses such as infective endocarditis or pneumonitis. If the chest pain is severe and abrupt in onset, it must be differentiated from concomitant ischemia or infarction of myocardial tissue. Interval changes in the ECG or changes in serum levels of cardiac muscle enzymes may be helpful. The presence of chest pain and pleural effusions may suggest pulmonary emboli, and a distinction between emboli and this syndrome is important.

Treatment & Prognosis

Most patients with this disease require no treatment other than analgesics for pain, eg, aspirin. Normally, the symptoms and fever can be controlled and will clear spontaneously over several weeks. At times, however, the patient will become sufficiently ill to require corticosteroid therapy for symptomatic relief. The clinical response to corticosteroids is usually prompt, but the symptoms may reappear after cessation of therapy. Excessive pericardial effusion may require drainage to prevent tamponade. Anticoagulant therapy is not recommended in postpericardiotomy syndrome because of the risk of hemopericardium.

ACUTE RHEUMATIC FEVER

Major Immunologic Features

- Follows group A streptococcal pharyngitis.
- Hereditary susceptibility to occurrence.
- Formation of antibodies to streptococcal cellular and extracellular antigens.
- Presence of cross-reactive antibodies that bind to various host tissues.
- Presence of lymphocytes that are cytotoxic to cardiac tissue.

General Considerations

Acute rheumatic fever is a collagen vascular disease that affects several organs in the human host. It is one of 2 nonpurulent complications of group A streptococcal infection in humans. Unlike nephritis (the other complication), rheumatic fever follows infection of the pharyngotonsillar tissue, but not the skin, and occurs only in predisposed individuals. Only 2–3% of a normal population appear to be susceptible. Rheumatic fever is encountered most commonly in children between the ages 5 and 15 years. Its incidence has declined steadily in Western countries but remains high in developing countries.

Clinically, acute rheumatic fever is manifested by inflammation of the joints, heart, brain, and skin. One or more of these organs may be involved in a patient with acute rheumatic fever. The frequency of organ involvement varies among patients. Cardiac involvement is the most serious manifestation of the disease.

Immunologic Pathogenesis

A. Mechanism of Tissue Injury: It was at first thought that tissue injury in acute rheumatic fever was due to (1) direct bacterial invasion of tissue or (2) the

effect of streptococcal toxins on the tissue. Lack of supportive evidence for these theories, together with the presence of a latency period of 2–3 weeks between the streptococcal infection and the manifestation of rheumatic fever, led to the current hypothesis that an immunologic mechanism is involved in the pathogenesis of tissue injury.

1. Cross-reactive antibodies–The role of "autoantibodies" in the pathogenesis of rheumatic carditis was supported by the finding of circulating antibodies to cardiac tissue as well as the presence of bound immunoglobulin in cardiac tissue of patients with rheumatic carditis. Subsequent studies revealed that antibodies raised in experimental animals against a number of streptococcal cellular antigens reacted with cardiac tissue and with other human tissues that are affected by the inflammatory process of rheumatic fever (Fig 27–1). Separate antigens, some associated with the M protein of the streptococcal cell wall and others present in the protoplasm membrane, manifest cross-reactivity with the sarcolemma of cardiac muscle and with human ventricular and skeletal muscle myosin. Recent studies have shown a structural homology between the M protein moiety and muscle tropomyosin. Immunologic cross-reactivity was also described (1) between the group-specific cell wall polysaccharide antigen and a valvular glycoprotein; (2) between an antigen in the streptococcal protoplasm membrane and the neuronal tissue of the caudate and subthalamic nuclei of the brain; and (3) between the streptococcal capsular hyaluronic acid and articular cartilage.

The identification of these cross-reactive antigens makes the theory of cytotoxic antibodies induced by the streptococcal organism an attractive one to explain tissue injury occurring in rheumatic fever. The presence of antiheart antibodies in patients with postpericardiotomy syndrome raises doubts about the specificity of these antibodies. However, this question was answered by recent studies demonstrating that heart-reactive antibodies in patients with rheumatic fever were absorbed by group A streptococci and by heart tissues, whereas the antibodies found in postpericardiotomy syndrome were absorbed only by cardiac tissue. A major issue that remains to be clarified is whether these antibodies which are found in the serum of more than half of patients with acute and chronic rheumatic fever as well as in patients with scarlet fever and in patients with poststreptococcal glomerulonephritis are cytotoxic. Although these antibodies bind to cardiac tissue, evidence that they are cytotoxic to these tissue is still lacking.

2. Cell-mediated immune mechanisms–Recent studies have provided evidence for the potential role of cell-mediated cytotoxicity in cardiac injury. Studies in laboratory animals have shown that lymphocytes from guinea pigs immunized with group A streptococci are cytotoxic to homologous cardiac cells grown in tissue culture. Lymphocytes from nonimmunized animals or from animals immunized with non-group A streptococci do not exhibit this cytotoxicity. Studies in humans have shown that peripheral blood lymphocytes from patients with rheumatic heart disease exhibit hyperreactivity in vitro to group A streptococcal antigens derived from strains associated with rheumatic fever but not to nephritogenic strains. More striking is the observation that peripheral blood lymphocytes from patients with acute rheumatic carditis are cytotoxic to human cardiac cells grown in tissue culture. Lymphocytes from patients without carditis do not exhibit a similar cytotoxicity. No cytotoxicity is expressed by the lymphocytes of patients with rheumatic carditis for noncardiac cells, such as skin cells. It is noteworthy that the cytotoxic effect of lymphocytes on the cardiac cells is blocked by the addition of homologous serum from the patient, suggesting that antiheart antibody is not cytotoxic but may serve a protective function.

B. Determinants of Susceptibility to Rheumatic Fever: A hereditary state of immunologic hyperreactivity has long been held to be the underlying cause of susceptibility to rheumatic fever. Prospective studies on the immunologic reactivity of patients who acquire rheumatic fever revealed that

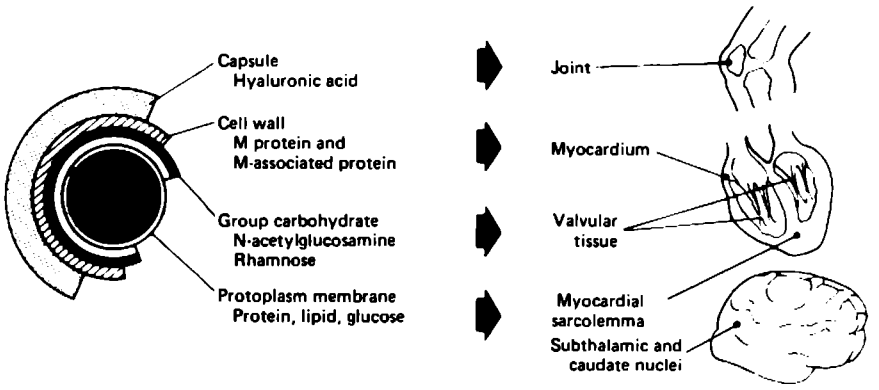

Figure 27–1. Streptococcal components and host tissues that share common antigenic determinants.

these individuals did show an immune hyperresponsiveness following immunization with bacterial *(Brucella)* antigens. Other studies failed to show an unusual response to other bacterial or viral antigens. Initial data also suggested that patients with rheumatic fever manifested an exaggerated response to some of the streptococcal extracellular antigens (streptolysin O). Subsequent studies using additional streptococcal extracellular antigens (DNase B, NADase) failed to reveal a significantly higher response to streptolysin O and NADase in patients with rheumatic fever when compared to patients with poststreptococcal glomerulonephritis.

In contrast to these findings, certain patients with rheumatic fever appear to exhibit an exaggerated response to a streptococcal cellular antigen, the group-specific carbohydrate. Patients with rheumatic valvular heart disease have been found to maintain elevated levels of antibody to this antigen for a long period following the initial attack of acute rheumatic fever. Antibody levels to this antigen in patients with rheumatic fever without cardiac involvement, or in patients with transient valvular involvement, and in patients with poststreptococcal nephritis decline to normal levels in about 2 years. In contrast, these antibodies remain elevated for about 10 years in the 80–85% of patients with persistent rheumatic valvular disease. Patients with congenital heart disease or patients with mitral valve prolapse of nonrheumatic etiology do not maintain elevated titers of this antibody. These observations suggest an altered immunoregulatory response to this streptococcal antigen in patients with rheumatic valvular disease.

Family studies have pointed to hereditary factors in susceptibility to rheumatic fever. These observations are now supported by studies which indicate that two B cell alloantigens are expressed on the leukocytes of most patients with rheumatic fever. This finding, together with data indicating a correlation between the immune response to streptococcal cell wall antigens and the inheritance of HLA haplotypes within families, provides a link between heredity and an immunologic mechanism in the pathogenesis of rheumatic fever.

Clinical Findings

A. Symptoms and Signs: The clinical manifestations of acute rheumatic fever are preceded by pharyngitis in about two-thirds of the patients. In half of those patients, the pharyngitis is mild and escapes clinical attention. A period of latency of about 2–3 weeks, during which the patient is asymptomatic, precedes the onset of the acute rheumatic symptoms.

1. Arthritis—Arthritis is the most common of the major manifestations, occurring in about 80% of patients. However, it is the least specific. The large joints are involved in migratory fashion. There is pain, redness, swelling, and warmth in the affected joints.

2. Carditis—Carditis occurs in about half of patients with acute rheumatic fever. The degree of cardiac involvement varies considerably. Some patients have mild carditis that is detected only by thorough examination. In others, the carditis is severe with involvement of endocardium, myocardium, and pericardium (pancarditis) and marked congestive failure. Severe pancarditis is potentially fatal. Carditis is associated with tachycardia at rest and when the patient is afebrile; with the presence of an apical systolic murmur of mitral insufficiency that commonly radiates to the axilla; and, in about 15% of cases, with the diastolic murmur of aortic insufficiency that is best heard at the second left intercostal space. The presence of a friction rub reflects pericardial involvement, which is almost always indicative of pancarditis in patients with acute rheumatic fever.

3. Sydenham's chorea—This manifestation is characterized by the presence of purposeless movement of voluntary muscles, aggravated when the patient is stressed. The choreiform movements diminish or disappear during rest and sleep. Emotional lability is often encountered. Rheumatic chorea occurs in about 15% of patients and is more common in white patients. Unlike arthritis and carditis, the period of latency preceding Sydenham's chorea averages 3–4 months or longer. Symptoms may last for 2 weeks or may persist for several months, resolving without neurologic residua.

4. Erythema marginatum—The rash characteristic of rheumatic fever is circular erythema surrounding normal skin. Lesions vary in size, averaging 2.5 cm in diameter, and are located on the trunk and proximal parts of the limbs, rarely on the face. This rash is seen in about 5% of patients, often escaping detection in black individuals.

5. Subcutaneous nodules—Rarely encountered nowadays, these lesions were seen in the past primarily in patients with chronic, severe carditis. The nodules are "pea-sized," firm, painless, and discrete. They are present on the extensor tendons overlying bony prominences.

6. Other manifestations—Findings in rheumatic fever include fever and abdominal pain, usually periumbilical, associated with nausea and sometimes vomiting. Occasionally, patients with these manifestations are thought to have acute appendicitis.

B. Laboratory Findings: The acute-phase reaction consists of leukocytosis, elevated erythrocyte sedimentation rate, and an elevated C-reactive protein level.

Electrocardiographic abnormalities present in patients with carditis include prolonged PR interval and ST–T changes. Prolongation of the PR interval may be seen independent of clinical evidence of carditis. Roentgenographic abnormalities include cardiomegaly and pulmonary venous congestion. Pulmonary infiltrates representing rheumatic pneumonitis are sometimes seen.

Antibody response to streptococcal antigens. Streptococcal pharyngitis is a universal antecedent to acute rheumatic fever. However, throat cultures are positive for group A streptococci in about half of patients with acute rheumatic fever. Evidence

Table 27–1. Recognized streptococcal antigens and commonly performed corresponding antibody tests.

Extracellular product	Test
Streptolysin O	ASO
Hyaluronidase	ASH
Streptokinase	ASK
Deoxyribonuclease B	Anti-DNase B
Nicotinamide adenine dinucleotidase	Anti-NADase
Cellular antigens	
M protein	Type-specific antibody
Group A carbohydrate	Anti-A CHO
Multiple antigens	Streptozyme

for group A streptococcal infection is best established by demonstrating a rise in antibodies to a number of streptococcal extracellular and cellular antigens or by finding significantly elevated levels of these antibodies. The recognized streptococcal antigens and the corresponding antibody tests commonly performed are listed in Table 27–1.

Elevated antibodies to one or more of the extracellular products are present in 92% of patients with acute rheumatic fever. However, an antibody response to one of these antigens occurs in 80–85% of patients, leaving about 15% with false-negative results if only one test is used. Thus, in patients with suspected rheumatic fever, it is preferable to perform additional antibody tests to other streptococcal antigens if the one test performed yields normal titers.

Antibody tests to the streptococcal cell wall antigens include the type-specific antibody test, which is performed to determine the presence of type-specific immunity to M protein; and the test for antibody to the group A carbohydrate. The latter test appears to be useful in patients with rheumatic valvular disease. Because of its prolonged persistence in these patients, it is useful in differentiating rheumatic from nonrheumatic valvular disease.

The Streptozyme test is an agglutination test utilizing latex particles coated with several streptococcal antigens. It is useful as a screening test but lacks the specificity of the other streptococcal antibody tests.

Immunologic Diagnosis

Although the antibody tests described above are useful in providing evidence for antecedent streptococcal infection, they are not diagnostic of rheumatic fever. In fact, no currently available test is diagnostic of this disease.

Differential Diagnosis

The diagnosis of acute rheumatic fever is still dependent on the utilization of the Jones criteria (Table 27–2). The finding of 2 major manifestations or one major and 2 minor manifestations outlined justifies a preliminary diagnosis of acute rheumatic fever. Confirmation requires provision of clinical, bacteriologic, or serologic evidence of antecedent streptococcal infection.

Because of its protean manifestations and the lack of a specific diagnostic test, acute rheumatic fever may mimic a variety of other illnesses. Acute rheumatic fever with arthritis should be differentiated from juvenile rheumatoid arthritis, systemic lupus erythematosus, or mixed connective tissue disease. The finding of antinuclear antibodies in the serum should allow for the differentiation of these collagen vascular disease. Serum sickness may mimic acute rheumatic fever. Reactive arthritis associated with some enteric infections or viral illnesses may also mimic this disease. Occasionally, patients with leukemia present with fever and polyarthritis.

Bacterial endocarditis or viral myocarditis should be considered in the differential diagnosis of rheumatic carditis. In patients with mitral insufficiency, mitral valve prolapse should be excluded as the primary condition. Patients with Sydenham's chorea often present a diagnostic problem, because evidence for preceding streptococcal infection is difficult to obtain. If the period of latency is quite prolonged, streptococcal antibodies may be close to the normal range when the patient presents with chorea. Tumors of the brain or degenerative disease of the central nervous system should be excluded.

Treatment

A. Acute Illness: Eradication of the streptococcal infection that preceded the rheumatic attack is achieved by administration of a therapeutic course of antibiotics. Give either long-acting penicillin G benzathine, 1.2 million units as a single injection; or oral penicillin, 250 mg 4 times daily. In patients allergic to penicillin, the alternative antibiotic is erythromycin, 50 mg/kg/d (maximum 1 g) orally in 4 divided doses.

1. General measures—Bed rest is usually self-imposed by the patient. Patients with severe arthritis, carditis, or chorea will prefer bed rest. Prolonged bed rest should not be imposed even on patients with cardi-

Table 27–2. Jones criteria (revised) for guidance in the diagnosis of rheumatic fever.*

Major Manifestations	Minor Manifestations
Carditis	**Clinical**
Polyarthritis	Previous rheumatic fever or rheumatic heart disease
Sydenham's chorea	Arthralgia
Erythema marginatum	Fever
Subcutaneous nodules	**Laboratory**
	Acute-phase reactions: Erythrocyte sedimentation rate, C-reactive protein, leukocytosis
	Prolonged PR interval

Plus

Supporting evidence of preceding streptococcal infection (increased ASO or other streptococcal antibody; positive throat culture for group A streptococci; recent scarlet fever).

The presence of 2 major criteria, or of one major and 2 minor criteria, indicates a high probability of the presence of rheumatic fever if supported by evidence of a preceding streptococcal infection.

*From: *Circulation* 1984;69:204A.

tis. Patients should gradually resume ambulation as soon as the carditis becomes stable. Resumption of normal activity should be encouraged as soon as possible in patients with minimal or moderate cardiac residua.

2. Arthritis—The arthritis of rheumatic fever is very responsive to salicylates. Lack of resolution of arthritis in 2–3 days following initiation of salicylate therapy is unusual. Salicylates should be given at a dose of 70–80 mg/kg/d in 4 divided doses. Salicylate levels of 20–25 mg/dL are therapeutic. Treatment should be continued for 2–3 weeks and gradually withdrawn over the following 2 weeks.

3. Carditis—Pancarditis and cardiac failure require prompt and careful attention. Prednisone, 1–2 mg/kg/d, is recommended for the treatment of pancarditis with or without cardiac failure. Prednisone is continued for 2–3 weeks and gradually withdrawn over the following 3–4 weeks. To avoid clinical rebound, salicylate therapy should be started when steroid withdrawal is initiated. Mild carditis is usually amenable to salicylate therapy.

Digitalization in patients with severe carditis and heart failure should be slow, since some patients with rheumatic carditis are very sensitive to digitalis. Some physicians prefer to digitalize with one-fourth or one-half the usual total digitalizing dose.

4. Sydenham's chorea—In most patients, the symptoms of chorea are fairly mild and self-limited, and bed rest and avoidance of stress are adequate management. In more severe cases, phenobarbital should be tried at an initial dose of 15–30 mg every 6 hours. The response of patients is quite variable.

B. Continuing Care: Patients who have recovered from an episode of acute rheumatic fever require continuing supervision, particularly in the period following the acute illness. The primary aim of such care should be to assure compliance with prophylaxis against recurrent streptococcal infections. Testing for penicillin should be performed on urine samples for patients on oral prophylaxis.

Antistreptococcal prophylaxis. Prevention of recurrent streptococcal infection by continuous administration of antibiotics should be enforced in all patients with rheumatic fever. Absence of cardiac involvement should not deter from this effort. Approved regimens include long-acting penicillin G benzathine, 1.2 million units once a month; oral penicillin, 250 mg twice daily; sulfadiazine, 500 mg twice daily; and erythromycin, 250 mg twice daily.

Patients with cardiac residua should also be instructed about the need for prophylaxis against bacterial endocarditis before undergoing surgery on the mouth, upper airway, gastrointestinal tract, or genitourinary tract.

Prognosis

Severe cardiac residua of rheumatic fever are rarely seen nowadays in Western countries. Mitral stenosis of rheumatic origin is uncommon. This change may be due to provision and enforcement of secondary prophylaxis. Prophylaxis programs have resulted in the prevention of additional cardiac damage from recurrent episodes of rheumatic fever. Studies report that 70% of patients with mitral insufficiency following an acute attack lose evidence of their mitral disease after 7–8 years if they are placed on and comply with continuous prophylaxis. This favorable prognosis should strongly encourage prophylaxis (probably lifelong) in all patients with rheumatic fever.

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Renal Diseases

28

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GLOMERULONEPHRITIS

Immunologically induced glomerulonephritis is estimated to be responsible for roughly one-half of instances of end-stage renal failure and its consequent mortality, morbidity, and expense. Antibody-associated mechanisms of glomerular injury can be broadly divided in terms of the physical state of the antigen involved—insoluble (tissue-fixed) and soluble (present in the body fluids). (See Table 28–1.) The antigen-antibody reaction most often leads to an immune deposition in the glomerulus, and the subsequent activation of mediators leads to foci of inflammation with damage to the surrounding tissue elements. Recently, it has been shown in model systems that the antibody attack can be more selective, with damage directed toward one glomerular cell type or another when cell membrane antigens are involved. Although human counterparts of this latter mechanism have not yet been defined with certainty, the possibility should be kept in mind. In contrast, human counterparts of the major antibody-induced glomerular immune deposit mechanisms are now well established.

Antibodies can form that react with fixed antigens in the kidney, which are present as a structural component or trapped there from some outside source. In humans, the major nephritogenic structural antigen or antigens identified to date are in the glomerular basement membrane (GBM), and antibodies reactive with the GBM are responsible for up to 5% of cases of human glomerulonephritis. Of potential importance in human glomerulonephritis, antibodies reactive with other glomerular antigens have been implicated in experimental glomerulonephritis. In some rabbits with spontaneous glomerulonephritis, an antibody reactive with one or more non-basement membrane glomerular antigens associated with the glomerular epithelial foot process has been found. Fixed structural subepithelial glomerular antigens associated with epithelial cell foot processes have been incriminated in the active and passive models of Heymann's nephritis in rats. We are currently studying a model in which mesangial cell membrane antigens can be used to induce an acute mesangiolytic lesion. Renal injury can also be induced experimentally by binding of antibody to "planted" antigens such as the lectin (concanavalin A; Con A) or other materials that have been previously bound to the GBM by physicochemical mechanisms such as reactions of cationic charges with the polyanionic glomerular capillary wall. Concomitant circulating immune complex formation (see below) from residual circulating soluble antigens in these planted antigen models may also contribute. The experimental observations about fixed or "planted" antigens has renewed clinical interest in determining if some facet of what has been regarded as immune complex disease may be related to glomerular trapping of antigen and subsequent local interaction with antibody. In most instances, the continuing presence of circulating antigen and the dynamic immune complex equilibrium make clear distinctions between these 2 modes of immune reactant accumulation quantitatively difficult to separate.

Glomerular injury can occur when antibodies react

Table 28–1. Immunopathogenesis of humorally mediated renal disease classified by the solubility of the antigen.

Solubility	Mechanism	Antigen	Condition
Insoluble or tissue-fixed antigens	Antibodies react with structural components of the kidney.	Glomerular basement membrane.	Glomerulonephritis.
		Tubular basement membrane.	Tubulointerstitial nephritis.
		Other glomerular wall antigens.	Experimental glomerulonephritis.
		Cell surface antigens.	Experimental glomerulonephritis.
	Antibodies react with antigens trapped or "planted" in the glomerulus.	Mesangial accumulations, immune complex components, lectins, cationic materials; possibly bacterial antigens, DNA.	Experimental glomerulonephritis. May contribute to human glomerulonephritis as well.
Soluble antigens	Antibodies react with antigens in the vascular compartment to form circulating immune complexes.	Exogenous antigens: drugs, products of infectious agents, etc.	Glomerulonephritis. Tubulointerstitial nephritis. Vasculitis.
		Endogenous antigens: nuclear antigens, tumor antigens, etc.	
	Antibodies react with antigens in the extravascular fluid near the site of antigen release.	Tubular antigens.	Experimental tubulointerstitial nephritis.

with soluble antigens in the circulation to form immune complexes, which subsequently accumulate in the glomerulus. Since immune complex formation is a dynamic process, continual modification of the deposited complexes by ongoing interaction with antibody and antigen or immune complexes from the circulation is to be expected. This continuing exchange with tissue deposits demonstrates the overlap or concomitant role of soluble and tissue-fixed antigens (as well as antibodies) in some forms of glomerulonephritis. Based on identification of nonglomerular exogenous and endogenous antigens and their antibodies in general, most cases of human glomerulonephritis appear to be caused by the immune complex mechanism, and this mechanism also appears to cause many spontaneous and experimentally induced cases of glomerulonephritis in animals. Antibodies reactive with soluble and insoluble antigens can cause tubulointerstitial renal injury as well as glomerulonephritis.

In contrast to humoral mechanisms of glomerular injury, little is known about the role of cellular immunity. Cellular sensitivity suggestive of a cellular immune response has been recognized in some forms of human glomerulonephritis. Models suggest that T cells can be identified in glomeruli in some experimental situations; however, any role for cellular immunity remains to be defined. There is increasing evidence of a basic defect at the cellular control level of the humoral response in some forms of nephritis. The presence of Ia-positive (HLA-DR-related) cells in the mesangial area of the glomerulus suggests a possible role for local immune regulation.

An important unanswered question is why only certain individuals develop glomerulonephritis in response to known or unknown antigens or causative factors. Immunogenetic factors undoubtedly play a role, with increasing numbers of associations between the HLA-DR system and glomerulopathies being recognized. The best-defined of these are HLA-DR2 with anti-GBM antibody-induced nephritis, HLA-DR3 (and the B cell antigen MT2) with primary membranous nephropathy, HLA-DR3 (and HLA-B8 which is in linkage disequilibrium with HLA-DR3) with SLE, and HLA-DR4 with mesangial IgA nephropathy. It is not known whether the actual gene products are involved in the generation of the disease or whether they are markers of another gene related to "disease susceptibility."

The glomerular injury caused by glomerular antibody accumulation results in large part from the action of immunologic mediation systems. The best-studied mediation systems in experimental glomerulonephritis are the complement and neutrophil systems. Complement activation generates biologically active components or fragments that initiate immune adherence, opsonization, histamine release, or leukocyte chemotaxis. It has been shown that the terminal complement membrane attack complex (MAC) is lytic for bacteria, erythrocytes, and nucleated cells. The MAC can insert into phospholipid membranes of erythrocytes, destroying normal osmotic and ionic permeability barriers and physically disrupting membrane structures. Recent studies have shown glomerular deposition of elements of the MAC (C5–C9) in both experimental models and human diseases, suggesting that the MAC may participate in the glomerular injury. In turn, in some situations, complement activation can serve to solubilize immune complex material. In most types of human glomerulonephritis, antibody deposition with complement components can be shown to be present in glomeruli by immunofluorescence microscopy, although the functional importance of these deposited components cannot be tested directly. Congenital deficiencies of the complement system may be associated with recurrent infections, the development of connective tissue disorders, or glomerulonephritis.

Neutrophils attracted by products of complement activation accumulate in the glomerular capillary loops in many forms of glomerulonephritis. There they can displace the endothelium and release enzymes and other materials, including reactive oxygen species, that may damage the basement membrane and induce proteinuria. Recent evidence that specific oxygen radical scavengers are able to modify experimental glomerulonephritis provides support for mediation by an oxidant in these lesions. In experimental animals, some types of glomerulonephritis have been shown to occur independently of complement and neutrophils, implicating other as yet undefined mediation pathways.

In addition to neutrophils and the complement system, macrophages, platelets, and humoral factors—including prostaglandins and the Hageman factor system—have been suggested as mediators of glomerular injury. Glomerular fibrin deposits have been found in glomerulonephritis, particularly in association with extracapillary crescent formation. In anti-GBM antibody and circulating immune complex-induced experimental glomerulonephritis, defibrination with ancrod (Malayan pit viper venom) has prevented glomerular fibrin deposition, crescent formation, and renal failure. However inflammatory infiltrates, endocapillary proliferation, proteinuria, and complement deposition were unaffected.

Macrophages are prominent in the glomeruli in some forms of experimental glomerulonephritis. The number of macrophages present correlates with the timing and extent of proteinuria and glomerular hypercellularity and the presence of crescent formation. Antimacrophage antisera have been used successfully to inhibit glomerular macrophage accumulation and in turn prevent some forms of experimental anti-GBM and immune complex-induced experimental glomerular injury. Morphologic evidence suggests that macrophages participate in glomerulonephritis in humans, particularly in crescentic glomerular lesions. Evidence for a role for platelets in the mediation of immunologic glomerular injury has not been fully evaluated. Studies are in progress on the involvement of the Hageman factor system and arachidonic acid metabolites in experimental glomerulonephritis. No proved role for the former has yet emerged, but pros-

taglandins of the E series can ameliorate the glomerular lesions of experimental immune complex glomerulonephritis and nephrotoxic nephritis.

Some forms of experimental and human glomerulonephritis are self-limiting, while others follow a progressive course. Factors that may influence disease chronicity include varied host immune responses, qualitative (alteration in matrix components) and quantitative (decreased renal mass) structural changes, the presence of hypertension, calcium and phosphate metabolism, and dietary protein intake. Irreversible glomerular damage is characterized by variable glomerular sclerosis and expansion of the mesangial matrix. Of interest in this regard is the observation in cell culture systems that macrophage supernatants or interleukin-1 can increase mesangial cell proliferation and collagen synthesis.

The immune complex and anti-GBM antibody mechanisms of glomerular disease may be indistinguishable from each other unless the patient is studied by immunopathologic means. In renal biopsy tissue, antibodies reactive with GBM have a characteristic linear configuration by immunofluorescence microscopy, whereas randomly deposited immune complexes have a granular pattern. In experimental models of other types of anti-glomerular antibody disease (involving nonclassic GBM antigen or antigens), noted earlier, the irregular distribution of the reactive antigen can result in irregular, granular antibody deposits that could be confused with those previously thought to be indicative of immune complex disease. Anti-GBM antibodies in the serum can now be detected by radioimmunoassay in almost all patients with the disease. In contrast, detection of circulating immune complexes has had less diagnostic value. Circulating immune complexes may be detectable easily only in acute cases of apparent immune complex glomerulonephritis or in patients whose glomerulonephritis is part of a systemic immune complex disorder such as lupus erythematosus.

When immunofluorescence is used to study renal biopsies from patients with glomerulonephritis, perhaps 10% of them show complement deposits in the absence of immunoglobulin. This observation, coupled with the finding of persistent hypocomplementemia in some patients with membranoproliferative histologic forms of glomerulonephritis, raises the possibility of yet another mechanism of glomerular injury. Such a mechanism could involve the "nonimmunologic" activation of immunologic mediator systems such as complement, a possibility that will be discussed in a subsequent section.

1. ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODY-INDUCED GLOMERULONEPHRITIS

Major Immunologic Features

- Linear deposition of immunoglobulin and often of complement occurs along GBM.
- Anti-GBM antibodies usually detectable in serum by radioimmunoassay; less often by indirect immunofluorescence techniques.

General Considerations

The nephritogenicity of antikidney antisera was noted in 1900 by Lindemann. Subsequent studies by Masugi et al amplified these early observations, and in the 1950s Krakower and Greenspon convincingly demonstrated that the major nephritogenic antigens of the kidney were in the GBM. Thereafter, the old term nephrotoxic antiserum was largely replaced by the more specific term "anti-GBM antibody."

In humans, anti-GBM antibodies are now implicated in the production of glomerulonephritis, glomerulonephritis and pulmonary hemorrhage (Goodpasture's syndrome), and occasionally clinical presentations indistinguishable from those of idiopathic pulmonary hemosiderosis. The possibility of central nervous system involvement caused by an interaction of these antibodies with the choroid plexus basement membrane has also been raised. In addition, some patients present with arthritic manifestations.

Immunologic Pathogenesis

Two forms of experimental anti-GBM antibody-induced glomerulonephritis have been demonstrated. First, anti-GBM antibodies can be induced by immunizing animals with GBM in adjuvant. The antibody can then be used to produce anti-GBM nephritis in normal recipients. For example, rabbit, sheep, or duck anti-rat GBM antibodies are often used to cause anti-GBM glomerulonephritis in rats. The resulting glomerular injury occurs in 2 phases. If sufficient quantities of anti-GBM antibodies are given (75 μg of kidney-fixing antibody per gram of kidney in the rat), immediate injury and proteinuria occur. If insufficient antibody is given to cause immediate injury, overt glomerulonephritis does not develop until the recipient has produced antibody reactive with the foreign immunoglobulin already bound to its GBM. The foreign immunoglobulin fixed to the GBM in this phase acts as a "planted" antigen. Quantitative differences in the numbers of antibody molecules required to induce immediate glomerular injury as well as the severity of the resultant glomerulonephritis have been equated with the antibody's ability to activate complement and to involve neutrophils. This situation has led to designation of the antibody as "dependent on" or "independent of" complement and polymorphonuclear leukocytes and indicates that separate mediators of immunologic injury must be utilized by the complement-independent antibodies. Similarly, one-fourth to one-third of patients with anti-GBM antibodies have no detectable complement fixed in the glomeruli, suggesting the possibility of complement-independent processes.

In the second form of experimental anti-GBM nephritis, some animals immunized with GBM in adjuvant (sheep are particularly susceptible) develop anti-GBM antibodies that cross-react with their own

GBM. Severe glomerulonephritis follows which can prove fatal to the sheep within 2–3 months. This lesion is similar in many ways to that of spontaneous anti-GBM glomerulonephritis in humans. Circulating anti-GBM antibodies are clearly pathogenic, because they can be used to transfer glomerular injury to normal lambs.

In research done in the mid 1960s, immunofluorescence of kidney sections from occasional patients with glomerulonephritis showed anti-GBM antibodies bound in a smooth linear pattern along the GBM. In 1967, Lerner and others demonstrated anti-GBM antibodies in the circulations of such patients. These investigators also isolated anti-GBM antibodies from the sera of these patients or eluted them from the nephritic kidneys and showed the antibodies' pathogenicity by using them to transfer anti-GBM glomerulonephritis to subhuman primates. The most convincing evidence of similar pathogenicity in humans came when glomerulonephritis was accidentally transferred to a renal transplant placed in a patient who had circulating anti-GBM antibodies.

The evidence suggests that anti-basement membrane antibodies are responsible for both the pulmonary and the renal injury in patients with Goodpasture's syndrome. In patients with Goodpasture's syndrome anti-GBM antibodies can be found bound to the alveolar basement membranes, and antibodies cross-reactive with the GBM can be eluted from the involved lung tissue. Experimentally, anti-GBM antibodies appear to cross-react with both the glomerular and alveolar basement membranes; however, lung injury is difficult to induce. Recently, it has been shown in rabbits that the binding of anti-lung antibodies to alveolar basement membrane is enhanced by prior lung injury. The infectious, toxic, or physiologic disturbances that often precede individual episodes of pulmonary hemorrhage in Goodpasture's syndrome may influence the accessibility of lung basement membrane antigen and subsequent antibody fixation.

Little is known about the events responsible for the induction of spontaneous anti-GBM responses in humans. As noted earlier, a genetic association is suggested with HLA-DR2. Materials cross-reactive with the GBM have been identified in the urine of animals and humans. Similar materials accumulate in the circulation after nephrectomy, suggesting that they represent basement membrane fragments released during basement membrane metabolism throughout the body and excreted in the urine. Immunization of rabbits with basement membrane antigens from their own urine can induce anti-GBM glomerulonephritis, suggesting the same potential in humans. In addition, noxious environmental or infectious insults to basement membranes (eg, in the lung) could induce anti-GBM antibody responses. Mercuric chloride administered to rats produces a transient anti-GBM antibody response. Both hydrocarbon solvent inhalation and influenza A2 infections have been associated with anti-GBM antibody-associated Goodpasture's syndrome in a few patients. The lung damaged in this way might also react more easily with anti-GBM antibodies formed for unrelated reasons. Development of anti-GBM antibody has also occasionally been associated with immunologic or ischemic renal injury and has occurred in patients with Hodgkin's disease. Differences in basement membrane antigens occur between individuals. Some individuals with hereditary glomerulonephritis lack the usual GBM antigens reactive with anti-GBM antibodies. Sometimes, anti-GBM antibodies can be induced in such individuals by transplantation of kidneys containing the normal antigens lacking in the recipient.

In virtually all instances, the spontaneous production of anti-GBM antibodies is self-limited (weeks to 1 or 2 years), suggesting that the immunologic stimulus is also transient and potentially identifiable. Most affected individuals have only one episode; however, one interesting patient had 3 distinct episodes of hemoptysis and mild glomerulonephritis over an 11-year period, with evidence of anti-GBM antibodies detected during the first and last episodes. The nature of the nephritogenic antigens within the GBM is at present only partially defined, but they appear to be noncollagenous glycoproteins containing heterosaccharide. Much current research, some utilizing basement membrane-producing tumors, concerns this area and suggests that the reactive antigen or antigens are in the noncollagenous extension of the type IV basement membrane collagen molecule. The inability of anti-GBM antibodies to react with the GBM from some patients with hereditary nephritis (some kindreds of Alport's syndrome), as noted above, indicates that these patients have an abnormal noncollagenous GBM component. The nephritogenicity of other components of the GBM, such as laminin, heparan sulfate, and GBM collagenous elements, are being investigated selectively using isolated fractions and polyclonal or monoclonal antibodies.

Clinical Features

Fewer than 5% of cases of glomerulonephritis in humans appear to be caused by anti-GBM antibodies. Most commonly, these antibodies induce proliferative crescent-forming histologic types of glomerular injury with rapidly progressive clinical courses (Table 28–2). One-half to two-thirds (depending on age and sex) of these patients with glomerulonephritis also have pulmonary hemorrhage and often respiratory failure, a condition referred to as Goodpasture's syndrome. It should be noted that although anti-GBM antibodies are probably the most frequent cause of Goodpasture's syndrome, immune complex mechanisms can induce a similar clinical picture; therefore, immunopathologic investigation is essential for correct diagnosis.

Anti-GBM antibody-induced diseases are more commonly identified in males in the second to fourth decades of life; however, either sex can be involved, and the disease affects children under age 5 through adults of advanced age. Indeed, a second grouping of cases occurs particularly in women over age 50. The

Table 28–2. Probable immunologic mechanisms of the principal histologic types of human glomerulonephritis (GN) and their systemic disease associations.

Histologic Classification	Probable Immunologic Mechanism	Primary (Idiopathic GN); Secondary (Systemic Disease–Associated) Types
Diffuse proliferative GN	"Immune complex" type "Anti-GBM" type	Idiopathic GN; SLE, chronic infections, thyroiditis, etc, GN; Goodpasture's syndrome
Diffuse proliferative GN (acute postinfectious)	"Immune complex" type (?"planted antigen")	Poststreptococcal GN and other infections (bacterial, viral, etc)
Crescent-forming diffuse proliferative GN	"Anti-GBM" type	GN only or Goodpasture's syndrome
	"Immune complex" type	Idiopathic GN; SLE, Wegener's granulomatosis, severe poststreptococcal GN, etc
	"No immunoglobulin" type (mechanism unknown)	Idiopathic GN; polyarteritis, glomerulitis, etc
Focal proliferative GN		
Mesangial IgA nephropathy	?"Immune complex" type (IgA antibodies or polymers)	Idiopathic GN (often "benign recurrent hematuria" syndrome); Henoch-Schönlein purpura, cirrhosis
Other	"Immune complex" type (other or multiple Ig classes)	Idiopathic GN; SLE, postinfectious, etc
Membranous GN	"Immune complex" type, ?"planted antigen" mechanisms (Autoantibody to glomerular wall antigens implicated in some experimental models)	Idiopathic GN; SLE, tumor-related, chronic infections, etc
Membranoproliferative ("mesangiocapillary") GN		
Type I (subendothelial deposits)	"Immune complex" type, plus ?alternative complement pathway involvement	Idiopathic GN (sometimes called "hypocomplementemic GN"); occasionally SLE, etc
Type II ("intramembranous dense deposit disease")	?Alternative complement pathway involvement with C3 in renal basement membranes	Idiopathic GN (sometimes called "hypocomplementemic GN"); sometimes associated with partial lipodystrophy
Focal glomerulosclerosis	Probably nonspecific accumulation of immunologic reactants within mesangium or GBM	Idiopathic GN; occasionally associated with heroin abuse, ?AIDS, ?relationship to recurrent minimal-change nephropathy
End-stage (chronic) GN	Advanced destruction initiated by any of the above mechanisms	Idiopathic GN; secondary to any of the above systemic disease associations
Minimal-change nephropathy	Unknown cause (hypotheses include lymphokine nephrotoxicity)	Idiopathic nephrotic syndrome; occasionally Hodgkin's disease, sometimes with atopic disease

first symptoms may be either renal or pulmonary or both, with onset occurring simultaneously or separated by as much as a year. In many patients, particularly those with Goodpasture's syndrome, flulike symptomatology precedes the onset of renal or pulmonary symptoms. It is often unclear if this is a true infectious illness or merely prodromal symptoms. Arthritis has also been a prominent early complaint in some (less than 10%) patients. Overall, about 75% of patients develop renal failure necessitating dialysis, although the outlook seems to be improving somewhat, probably as a result of early diagnosis and more aggressive treatment. As experience with the disease increases and better diagnostic methods evolve, it has become evident that a milder form does occur. This milder form, however, accounts for fewer than 15% of cases. In a few patients, the pulmonary features of the disease predominate or are the sole clinical manifestation. Anti-GBM antibodies appear to be responsible for an as yet undetermined percentage of cases of so-called pulmonary hemosiderosis. Nephrotic syndrome is unusual in anti-GBM glomerulonephritis, probably because renal failure supervenes before sufficient urinary protein spillage has occurred.

Immunologic Diagnosis

Patients with rapidly progressive glomerulonephritis or Goodpasture's syndrome should be suspected of having anti-GBM antibodies. However, immune complex disease and other undetermined causes are responsible for half or more than half of cases of rapidly progressive glomerulonephritis. A diagnosis of anti-GBM glomerulonephritis is based on identifying anti-GBM antibodies by at least 2 of the 3 following means: (1) linear deposits of immunoglobulin along the GBM seen by immunofluorescence, (2) elution of anti-GBM antibodies from renal tissue, and (3) detection of circulating anti-GBM antibodies.

By immunofluorescence, anti-GBM antibodies appear as linear deposits of IgG and infrequently IgA or IgM along the GBM (Fig 28–1). Irregular IgM deposits along the GBM are present in less than half of the kidneys studied. Linear deposits of immunoglobulin are also frequently present along the tubular base-

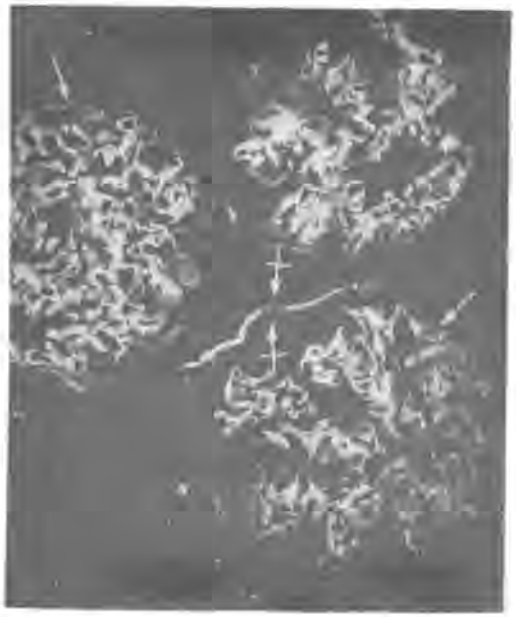

Figure 28–1. Smooth linear deposits of IgG (arrows) representing anti-GBM antibodies are seen outlining the GBM of 3 glomeruli from a young man with Goodpasture's syndrome. The antibody also had reactivity with Bowman's capsule (opposed hatched arrows). (Original magnification × 160.)

ment membrane (TBM) (see later section). The linear deposits of immunoglobulin are accompanied by linear or irregular deposits of C3 in about two-thirds of these kidneys. Fibrin-related antigens may be striking in areas of extracapillary proliferation and crescent formation. When C3 is present, it is usually accompanied by deposits of other components of the classic complement pathway. Care must be taken in basing the diagnosis on immunofluorescence study alone. Nonimmunologic accumulations of IgG are sometimes observed in a linear pattern along the GBM, prticularly in kidneys from patients with diabetes mellitus, in kidneys obtained at autopsy, in kidneys perfused in preparation for transplantation, and in some reasonably normal kidneys. These occasional "false-positive" kidneys indicate the need for confirming diagnoses by eluting anti-GBM antibodies or detecting them in the circulation.

Anti-GBM antibodies can be dissociated from renal or lung tissue by elution in acid buffers or buffers containing chaotropic ions (KI, KSCN, etc), which can dissociate hydrophobic and ionic bonds. The eluted immunoglobulin can then be recovered and tested for anti-GBM reactivity in vitro by indirect immunofluorescence or radioimmunoassay or in vivo by injection into subhuman primates. The ability to elute GBM-reactive antibodies from renal tissue confirms the specificity of immunofluorescence observations and establishes a clear-cut diagnosis.

Anti-GBM antibodies can be sought in the circulation by using indirect immunofluorescence, hemagglutination, or, more recently, radioimmunoassay. About 80% of patients with anti-GBM antibody-induced Goodpasture's syndrome and 60% of patients with anti-GBM antibody-induced glomerulonephritis alone have anti-GBM antibodies detectable by indirect immunofluorescence. By radioimmunoassay, almost all of both groups are positive when serum is available early in the course of the disease. Patients may have pulmonary hemorrhage at any time during the period of their anti-basement membrane antibody production, apparently unrelated to the amount of circulating anti-GBM antibody detected by radioimmunoassay. This suggests that nonimmunologic factors such as fluid overload and infection may contribute to the lung injury. Nephrectomy has no immediate effect on the levels of circulating anti-GBM antibodies in most patients, which suggests that damaged kidneys have little or no residual immunoabsorptive properties.

A group of patients with rapidly progressive, crescentic glomerulonephritis exists in whom neither anti-GBM antibodies nor immune complexes are detected (Table 28–2). These patients may share clinical features with anti-GBM antibody-induced glomerulonephritis, including flulike prodromes, arthralgia, and occasionally hemoptysis. Diffuse endocapillary proliferation and extensive crescent formation are observed without the detection of immunoglobulin or complement by fluorescence microscopy or evidence of electron-dense deposits by electron microscopy. However, extensive deposition of fibrin can be found in relation to the crescents. The pathogenesis of this lesion is unknown.

Treatment

No immunologically specific treatment is available. Corticosteroids are thought to be helpful in the management of acute pulmonary hemorrhage in patients with anti-GBM antibody-associated Goodpasture's syndrome. The variable duration and intensity of the transient anti-GBM response must be considered in evaluating any therapeutic regimen, and occasional patients have recovered adequate renal function "spontaneously" even after being on dialysis for short periods of time. Approaches have recently been employed to hasten the disappearance of antibodies as a therapeutic modality. Repeated and intensive plasmaphereses in conjunction with immunosuppression are employed to remove circulating anti-GBM antibodies and to impair their production. Initial results are promising if the combined therapy is instituted before irreversible renal damage has taken place, in those patients without oliguria, and in patients with fewer circumferential crescents. A recent controlled trial has shown only minimal improvement in patients undergoing plasmapheresis and immunosuppression compared to similarly immunosuppressed controls, with the ultimate outcome influenced by the severity of injury at the initiation of therapy. Initial improvement is not sustained in all patients, so that long-term follow-up will also be needed.

Although nephrectomy was suggested by some in the past as being helpful or even lifesaving in the management of severe pulmonary hemorrhage in Goodpasture's syndrome, results have been disappointing, and nephrectomy is now rarely advised. Several nephrectomized patients have continued to manifest pulmonary hemorrhage, sometimes leading to death. Nephrectomy may or may not facilitate termination of production of anti-GBM antibody, and it is thus unclear whether it should be considered even as a last resort. Nephrectomy obviously precludes the occasional spontaneous recovery of renal function.

Circulating anti-GBM antibodies can transfer glomerulonephritis to a transplanted kidney. The severity with which the anti-GBM disease recurs in the transplant seems to relate in a general way to the level of antibody present at the time of transplantation and is certainly influenced and modified by the intensive immunosuppression the transplant recipient receives. Quantities of anti-GBM antibodies insufficient to cause histologic change may be detected by immunofluorescence in some recipients. It is therefore advisable to postpone transplantation in patients with anti-GBM antibody-associated glomerulonephritis until circulating anti-GBM antibodies are absent or greatly reduced. The mean duration of the anti-GBM antibody response measured with today's sensitive radioimmunoassays is about 12 months (range, a few weeks to 3 years). Immunosuppression and plasmapheresis appear to hasten the disappearance of the anti-GBM response.

2. IMMUNE COMPLEX & RELATED GLOMERULONEPHRITIDES

Major Immunologic Features

- Granular deposition of immunoglobulins and complement occurs in the glomeruli.
- Circulating immune complexes may be detectable in some cases.

General Considerations

In 1911, von Pirquet recognized the relationship between the immune response and the symptoms of serum sickness. Dixon and others demonstrated, in the 1950s, that immune complexes composed of antigen and antibody were the toxic products responsible for the tissue injury of individuals with serum sickness. It has become widely accepted that the demonstration by immunofluorescence of granular deposits of immunoglobulin in the glomerulus can usually be interpreted as evidence of immune complex-mediated nephritis. It must be kept in mind that the direct reaction of antibody with irregularly distributed fixed or "planted" antigens, as noted earlier in animal models, could be confused with immune complex deposits by immunofluorescence, thereby stressing the need for identification of the antigen-antibody systems involved.

Immunologic Pathogenesis

The principles of immune complex-induced renal injury are best understood by examining the events that accompany acute and chronic serum sickness in rabbits. To induce acute serum sickness, one gives rabbits large amounts of foreign proteins such as bovine serum albumin (BSA), 250 mg/kg. The BSA rapidly equilibrates with the intra- and extravascular fluids and then disappears at a rate governed by its catabolic half-life. Antibody production is initiated, and after 4–5 days, sufficient antibody is present to combine with the circulating antigen to be detected in circulating immune complexes. Since antigen is present in great excess, the complexes remain small and continue to circulate. As antibody production increases, the complexes increase in size and after 10–12 days are eliminated by the mononuclear phagocytic system. During the process of eliminating the immune complexes, small amounts (mean, 18 μg) of BSA in immune complex form are deposited in the kidney, inducing a severe but transient endocapillary proliferative glomerulonephritis with macrophage accumulation manifested clinically by heavy proteinuria. Immune complexes are also deposited in other vascular beds, inducing conditions such as arteritis and synovitis.

Evidence for deposition of circulting immune complexes is substantial in this model. There is no evidence that BSA is handled differently than the rabbit's own albumin by the kidney before immune complexes form. Since antigen excess is present, all antibody is immediately complexed and must reach the kidney in immune complex form. In addition, only complexes of a certain size (larger than 19S) are deposited in the kidney, and then only if vasoactive amine release has occurred. After antigen has been eliminated from the circulation, free antibody appears, combining with and masking the antigen complexed within the glomerulus. This demonstrates that tissue-bound immune complexes are in equilibrium with antibody and with antigen from the circulation; the composition of the glomerular-bound immune complexes is then determined at any point in time by the relative concentrations of antigen or antibody in the circulation.

The mechanisms responsible for mediating injury in acute serum sickness have been poorly understood until recently. Depleting an animal of complement and of polymorphonuclear leukocytes prevents arteritis but does not prevent glomerular injury. Antimacrophage serum, however, does abrogate the histologic and functional lesion.

If the rabbits are given BSA repeatedly in amounts to balance antibody production, chronic serum sickness glomerulonephritis can be induced. A spectrum of glomerulinephritis develops in this model that resembles many of the histologic types of glomerulonephritis found in humans. During the 6–8 weeks of daily intravenous BSA injection required for the development of chronic serum sickness glomerulonephritis, only small amounts of radiolabeled BSA accumulate in the glomerular immune deposits in the

kidney. When viewed by immunofluorescence, the deposition is confined largely to the mesangium, where it induces hypercellularity and macrophage accumulation. After this time, the deposition increases to about 0.5% of the daily injected dose (10–200 mg), and localization of the deposits changes from the mesangium to the peripheral GBM, with occasional deposits observed in extraglomerular renal structures (tubular basement membrane [TBM], interstitium, and peritubular capillaries) and other tissues as well. A wide variety of glomerular histologic changes are induced by the immune complex accumulation, apparently governed in part by the quantity and rate of this deposition. Rabbits with poor immune responses and with low levels of antibody are given correspondingly small amounts of antigen; as a result, they form only minimal amounts of immune complexes. Such rabbits tend to develop membranous glomerulonephritis with prolonged immunization. In contrast, active antibody producers given large amounts of antigen form large amounts of circulating immune complexes and have more proliferative (often crescent-forming) histologic changes.

The half-life for disappearance of the bound antigen from the kidneys of rabbits with chronic serum sickness glomerulonephritis is about 5 days. It has been possible to hasten the disappearance of the renal-bound immune complexes by deliberately creating huge antigen excess in a rabbit's circulation. This treatment dissolves the glomerular-bound immune complexes (detected by immunofluorescence and electron microscopy), and recovery follows if the excess antigen is given before irreversible glomerular changes develop. Administration of a huge antigen excess also terminates the antibody response, preventing formation of additional immune complexes. The multiple therapeutic benefits of this treatment may eventually be useful in humans.

Although still incompletely understood, several factors have been identified that may influence the tissue localization of immune complexes. In thinking about these factors, it must be remembered that the dynamics of immune complex formation render the complex subject to continual modification as shifts in the relative concentration of either antigen or antibody occur. Once localization has begun, antigen, antibody, or their complexes can interact at the site. This local interaction has made it very difficult to quantitate the exact contribution of the several factors that have been recognized. The glomerulus seems to be a uniquely susceptible site for immune complex accumulation, probably related in part to its function as an arterial capillary filter with a fenestrated endothelial lining.

Factors influencing immune complex accumulation can be viewed in terms of the characteristics and quantity of immune complexes reaching the glomerulus and local factors within the glomerulus itself. The blood flow or its alteration is important, as is the systemic clearance of immune complexes by the mononuclear phagocytic system; both represent major factors determining the presentation of immune complexes to the glomerulus. It has been demonstrated that patients with autoimmune disease and tissue deposition of immune complexes have defective Fc receptor-mediated phagocytic clearance. Experimentally, immune complexes that have been altered to remove Fc and C3 reactivity are cleared slowly and have enhanced glomerular accumulation.

The size of the circulating immune complex, which is determined by the relative antigen:antibody ratio and size, valence, and nature of the antigen as well as the antibody class and the antibody affinity, influences the fate of the complex. Great antigen excess produces small complexes that are not particularly nephritogenic. In great antibody excess, large, often insoluble complexes form which are rapidly removed from the circulation by the mononuclear phagocytic system and are not available for vascular localization. If such complexes are purposely created in the circulation leading to the kidney, they do localize in vessels and mesangial areas but are rapidly removed, in contrast to those formed during a period when the antigen:antibody ratio is more balanced.

Local factors that affect immune complex deposition in the glomerulus include features of the glomerular capillary wall such as permeability and electrical charge and the composition of the immune complex. The degree of dynamic remodeling of the complex also appears to be particularly important. Vasoactive substances released as part of the immune response—in particular that involving mast cell-bound or basophil-bound IgE—may enhance vascular permeability and potentially immune complex localization. Indeed antihistamine and antiserotonins have been suggested to decrease immune complex deposition in rabbits with acute serum sickness. The physicochemical properties of the antigen, antibody, and the resultant antigen-antibody complex have a bearing on their affinity for the highly charged glomerular capillary wall. Cationic antigens seem to preferentially accumulate at the anionic sites of the subepithelial aspect of the GBM. Such an affinity for the glomerular capillary wall brings into question the relationship between immune complexes being formed in situ through independent antigen-antibody interaction in the glomerular capillary wall and immune complexes that are deposited in the wall as a consequence of circulating immune complex formation. One must also consider the dynamics of immune complex assembly, ie, dissolution and reformation, when evaluating the accumulation and transit of the complexed material into and through the glomerular capillary wall. Varying ratios of antigen and antibody occur in the daily injection models of chronic serum sickness as well as in human immune complex disease. The interchange of antigen and antibody with previously deposited immune complexes is influenced by location, degree of inflammation, affinity of antibody, and physicochemical factors. The C3b receptor present on glomerular epithelial cells in humans conceivably could influence the remodeling process. In turn, C3b can serve to solubilize immune complex material. Secondary deposits of anti-im-

munoglobulin antibodies such as rheumatoid factor and anti-idiotypic antibodies may also alter free interchange between the primary antigen-antibody system.

Many chronic infectious processes, exemplified by viral infections, provide sufficient exogenous antigens to eventually provoke circulating and potentially nephritogenic immune complex formation. The response of mice to chronic lymphocytic choriomeningitis virus is considered the prototype of viral immune complex mechanisms. Mice infected at birth with lymphocytic choriomeningitis virus maintain lifelong infection and have thought by some to be immunologically unresponsive to the virus. Antibodies bound to circulating viral antigens can be detected, however, by immunoprecipitation. The antibody is completely complexed with the antigen and is undetectable by commonly employed immunologic techniques. The complexes lodge in the glomeruli, beginning shortly after birth in mice infected in utero. Similarly, antigens from murine leukemia virus, Aleutian mink disease virus, and infectious equine anemia virus lead to immune complex glomerulonephritis in mice, mink, and horses, respectively.

Endogenous or self antigens that are involved in producing autoimmune antibody may also cause immune complex diseases. At least 3 strains of mice, of which the New Zealand black and the New Zealand black-white hybrid are the best known, develop antibodies reactive with nuclear materials such as DNA. These antibody responses are associated with the development of a systemic lupus erythematosus-like immune complex disease. The mice have various abnormalities in their immune systems that can be influenced by different accelerating factors, resulting in loss of the usual resistance to autoantibody formation. The immune complex deposits are not confined to the glomeruli but may involve extraglomerular renal sites (TBM and interstitium) and extrarenal structures such as the choroid plexus and coronary vessels as well. Antibodies also form that are reactive with retroviral antigens such as gp70, forming circulating and deposited immune complexes. Other endogenous antigens, such as thyroglobulin, erythrocyte surface antigens, and histocompatibility antigens can cause immune complex glomerulonephritis in experimental animals.

An ever-increasing number of antigen-antibody systems are being identified in immune complex glomerulonephritis in humans (Table 28–3). As in animals, the antigens in humans can be divided into exogenous or foreign and endogenous or self antigens. Administration of foreign proteins used for passive immunization as well as inoculations and drugs can result in serum sickness-like immune complex diseases. Infectious agents provide the largest number of antigens identified to date in humans. Streptococcal antigens have been identified in some patients with poststreptococcal glomerulonephritis, staphylococcal antigen in children with infected ventriculoatrial shunts, enterococcal antigen in glomerular immune complex deposits of a patient with subacute bacterial endocarditis, and *Salmonella* antigen in glomerulonephritis associated with typhoid fever. In some instances, the bacterial antigens have been formed only early in disease and are not clearly associated with antibody, suggesting some element of local trapping for subsequent antibody interaction. *Treponema pallidum* antigens were recently identified in a patient with syphilis-associated glomerulonephritis. Chronic parasitic infections also provide antigens for immune complex formation. Both *Plasmodium malariae* and *Plasmodium falciparum* antigens have been found in patients with malaria and glomerulonephritis, and patients with congenital toxoplasmosis and glomerulonephritis have immune complexes containing *toxoplasma gondii* antigens. Filariasis and schistosomiasis are also associated with immune complex glomerulonephritis—again presumably related to antigens from these parasitic infections. Hepatitis B, measles, and Epstein-Barr viral antigens in patients with hepatitis, subacute sclerosing panencephalitis, and Burkitt's lymphoma, respectively, can also contribute to neph-

Table 28–3. Antigen-antibody systems known to cause or strongly suspected of causing immune complex glomerulonephritis in humans.

Antigens	Clinical Condition
Exogenous or foreign antigens	
Iatrogenic agents	
Drugs, toxoids, foreign serum	Serum sickness, heroin nephropathy(?), gold nephropathy(?), etc
Infectious agents	
Bacterial: Nephritogenic streptococci, *Staphylococcus albus* and *aureus, Corynebacterium bovis,* enterococci, *Streptococcus pneumoniae,* Propionibacterium acnes, *Klebsiella pneumoniae, Yersinia enterocolitica, Treponema pallidum, Salmonella typhi, Mycoplasma pneumoniae*	Poststreptococcal glomerulonephritis, infected ventriculoatrial shunts, endocarditis, pneumonia, yersiniasis, syphilis, typhoid fever, pneumonia
Parasitic: *Plasmodium malariae, Plasmodium falciparum, Schistosoma mansoni, Echinococcus granulosus, Toxoplasma gondii*	Malaria, schistosomiasis, toxoplasmosis, hydatid disease
Viral: Hepatitis B, retrovirus-related antigen, measles, Epstein-Barr virus, cytomegalovirus	Hepatitis, leukemia, subacute sclerosing panencephalitis, Burkitt's lymphoma, cytomegalovirus infection
Fungal: *Candida albicans*	Candidiasis
Perhaps others as yet undetermined	Endocarditis, leprosy, kala-azar, dengue, mumps, varicella, infectious mononucleosis, Guillain-Barré syndrome, ?AIDS
Endogenous or self antigens	
Nuclear antigens	Systemic lupus erythematosus
Immunoglobulin	Cryoglobulinemia
Tumor antigens	Neoplasms
Thyroglobulin	Thyroiditis

ritogenic immune complex formation. As might be expected, patients with AIDS also have evidence of immune deposition in their glomeruli. The reason that only certain individuals will develop nephritis when exposed to infectious agents may be related to a genetic "disease susceptibility" mediated through defective handling of either the antigen or the immune complexes generated during the infection, or perhaps more likely through an inappropriate antibody response. Finally, in most cases of presumed human immune complex glomerulonephritis, the antigen-antibody systems are unknown. The obvious difficulty is in screening for antigens for which no clinical clues are available. Some of these difficulties may be related to the possible involvement of multiple or ubiquitous antigens.

Endogenous antigens leading to immune complex formation are best exemplified in the glomerulonephritis of patients with systemic lupus erythematosus, who form antibodies reactive with a variety of nuclear materials. Their immune responses can lead to nephritogenic immune complex formation, and their disease activity most clearly relates to the presence of native DNA antigen-antibody complexes. Recent studies in animals have suggested that DNA can bind to GBM, leading to the speculation that some deposits may form locally. Circulating immune complex localization, however, remains the favored immunopathogenic mechanism for this disease. Rheumatoid factors and cryoglobulins may contribute to the phlogogenic glomerular accumulations in SLE.

Immunoglobulin aggregates or complexes such as those seen in essential cryoglobulinemia may also cause glomerular injury. Thyroglobulin-antithyroglobulin immune complexes have been identified in glomeruli of patients with thyroiditis, and radiation-induced thyroid damage can release sufficient thyroglobulin to shift the ratio of circulating antigen and antibody so that nephritogenic immune complexes form. Immune complexes containing renal tubular brush border antigen have been reported in a few patients; however, the tubular antigen system remains controversial. We have recovered anti-brush border antibodies in the eluate of one infant with membranous nephropathy; however, the tubular antigen-antibody system appears to be rare. Finally, antigens associated with neoplasms have also been identified in glomerular immune complex deposits of patients with neoplasia.

The difficulty in distinguishing "immune complex" from "planted antigen" mechanisms has already been discussed, including the possibility that these 2 systems may coexist in an individual patient. Therefore, implication of these pathogenetic mechanisms in specific histologic types of human glomerulonephritis must still be regarded as speculative.

Clinical Features

The granular, irregular, presumed immune complex-related deposition of immunoglobulin (and complement), both in the glomerular capillary wall and in the mesangium, appears to be responsible for over 75% of cases of human glomerulonephritis and encompasses a wide range of histologic and clinical presentations (Table 28–4). Primary immune complex glomerulonephritis (ie, patients without identifiable systemic disease) is usually classified histologically into diffuse, focal, and crescent-forming proliferative types; membranoproliferative (mesangiocapillary) glomerulonephritis; membranous glomerulonephritis; and end stage glomerulonephritis. The glomerulonephritis associated with systemic diseases such as Henoch-Schönlein purpura, SLE, and subacute infective endocarditis is often of a proliferative type, though several histologic variants occur.

The clinical features of these various forms may include any or all of the possible manifestations of glomerular damage. Proteinuria may be mild, moderate, or severe; nephrotic syndrome occurs when urinary protein loss exceeds the body's capacity to completely replace it, after which serum oncotic pressure decreases and edema develops. Nephrotic syndrome most frequently accompanies focal glomerulosclerosis and membranous glomerulonephritis. Hematuria is more common in patients with proliferative or membranoproliferative histologic findings. The presence of red blood cell casts in the urinary sediment suggests an acute phase of glomerular inflammation. Hypertension can be present from the outset of any of these diseases or may appear later if the nephritis progresses.

Chronic glomerulonephritis may follow any form of immune complex glomerular injury, although the likelihood of such progression can be related to the particular histologic type. For instance, the acute diffuse proliferative lesion of poststreptococcal glomerulonephritis frequently appears to resolve completely, especially in children, even though chronic glomerulonephritis sometimes develops later after many years of apparent good health. A fluctuating course with exacerbations and remissions may occur in systemic lupus nephritis, Henoch-Schönlein nephritis, and focal proliferative glomerulonephritis, whereas in membranoproliferative and some other forms of proliferative glomerulonephritis a steady progression to chronic renal failure is typical, sometimes taking many years. Crescent-forming proliferative lesions are usually associated with rapidly progressive glomerulonephritis, which destroys the kidneys within a few weeks to months; this form of immune complex disease in particular may be clinically and morphologically indistinguishable from anti-GBM-mediated nephritis. Similarly, immune complex mechanisms can induce a clinical picture much like that of anti-GBM antibody-induced Goodpasture's syndrome.

Hypocomplementemia occurs frequently in certain forms of immune complex-mediated glomerulonephritis, especially SLE and infection-associated glomerulonephritis (poststreptococcal, endocarditis, infected ventriculoatrial shunts, etc). It is also common in membranoproliferative (mesangiocapillary) types of glomerulonephritis, a disease group incorporating at least 2 varieties of nephritis with differing

Table 28–4. Generalizations regarding morphologic and clinical features of immune complex and related forms of glomerulonephritis in humans.

Morphology	Clinical Features
Proliferative glomerulonephritis	
Diffuse proliferative glomerulonephritis; diffuse hypercellularity, electron-dense glomerular deposits.	Proteinuria, hematuria; nephrotic syndrome hypertension may be present. Onset and course variable but often progressive to renal failure.
Postinfectious glomerulonephritis; as above with subepithelial electron-dense "humps."	Generally poststreptococcal; acute onset of edema, oliguria, hypertension, with proteinuria, hematuria, and red blood cell casts in urinary sediment. Usually resolves in children. Hypocomplementemia usual, lasting 6–8 weeks.
Diffuse proliferative, crescent-forming glomerulonephritis; diffuse hypercellularity with extracapillary crescent formation; electron-dense deposits in the GBM or mesangium (or both).	Rapidly progressive renal failure, or may be anuric or oliguric from onset. Proteinuria, hematuria, red blood cell casts in urinary sediment. Nephrotic syndrome unusual. Sometimes microangiopathic hemolytic anemia.
Focal or segmental proliferative glomerulonephritis; focal and segmental hypercellularity; often confined to the mesangium; electron-dense deposits in the GBM or mesangium (or both).	Proteinuria or hematuria frequent; episodes of gross hematuria may accompany intercurrent respiratory infections (syndrome of "benign recurrent hematuria"); otherwise may be asymptomatic. May occasionally progress to renal failure.
Membranous glomerulonephritis	
Thickening of the GBM with few or no proliferative changes; subepithelial "spikes" with silver stains; diffuse subepithelial electron-dense deposits.	Proteinuria, often with nephrotic syndrome. Slow progression in one-third to renal failure with remission in one-third.
Membranoproliferative glomerulonephritis	
Mesangial proliferation and hypertrophy with interposition between endothelium and thickened GBM; at least 2 electron microscopic variants on the basis of dense deposits: subendothelial (type I) and intramembranous (type II).	Proteinuria, hematuria; often nephrotic syndrome or hypertension. Persistent hypocomplementemia in most cases. Common in children and young adults; usually progresses to renal failure.
End-stage (chronic) glomerulonephritis	
End-stage renal architecture, hyalinized glomeruli, extensive tubulointerstitial damage; electron-dense glomerular deposits may be present.	Proteinuria, nypertension frequent. Renal failure progressing to uremia. End stage of many morphologic forms of glomerulonephritis.

pathogenetic mechanisms. Both are characterized morphologically by glomerular capillary wall thickening with varying degrees of mesangial expansion and interposition between endothelium and GBM. However, type I is an etiologically heterogeneous group with subendothelial immune deposits ultrastructurally and circulating immune complexes in the sera of some patients. Type II, or "dense deposit" disease, appears to be a generally uniform disease identified by a distinctive electron-dense transformation of the GBM (and TBM). In about 10% of cases, it may be associated with partial lipodystrophy. Additional morphologic variants of membranoproliferative glomerulonephritis have also been described. In type II, chemical analysis of the basement membrane does not suggest the accumulation of a non-basement membrane component but rather an increase in sialic acid-rich basement membrane glycoproteins. Evidence suggests that type I is an immune complex disease with immunoglobulin deposits and complement component deposits consistent with classic complement activation. In type II, immunoglobulin deposits are usually undetectable, but complement component accumulations consistent with alternative complement pathway activation are seen.

A serum factor capable of activating the alternative complement pathway is present in many patients with membranoproliferative glomerulonephritis, particularly type II. This factor, termed nephritic factor (NF), has been shown to be an immunoglobulin with immunoconglutinin properties capable of reacting with activated components of the alternative complement pathway—specifically, the bimolecular complex of C3b and activated factor B, stabilizing its C3 convertase activity. A similar factor has been found in patients with partial lipodystrophy with or without membranoproliferative glomerulonephritis. Occasionally, other C3 activation factors have been noted. No immunopathogenic role for NF has been established, and experiments to purposefully activate complement chronically in animals have not caused glomerular injury. Finnish Landrace sheep develop a histologic lesion similar to membranoproliferative glomerulonephritis in the absence of NF. Placental transfer of NF has occurred. NF is relatively species-specific, so that attempts to transfer disease to subhuman primates have not been successful. Membranoproliferative glomerulonephritis, particularly type II, frequently recurs in renal allografts but is unrelated to levels of NF.

Immunologic Diagnosis

The diagnosis of immune complex glomerulonephritis is based on finding granular deposits of immunoglobulin, usually accompanied by complement, in the glomeruli. This is, of course, only presumptive evidence until the antigens can be identified, since, as

has been mentioned, direct reactions of antibodies with irregularly distributed structural or planted antigens in the glomerulus could result in a similar pattern of deposit. The immunoglobulin most commonly found in these deposits is IgG, with IgA or IgM occasionally predominating (Table 28–5). The glomerular immune complex deposition may diffusely involve all capillary loops in membranous or diffuse proliferative glomerulonephritis (Fig 28–2A). In focal glomerulonephritis, the deposits tend to involve only segments of the glomerular capillary wall but may be more widespread than expected from the focal nature of the histologic change. In some patients, the immune complex deposition appears to be confined to the mesangium, usually causing only mild histologic and clinical evidence of glomerular damage (Fig 28–2B).

The class of immunoglobulin identified in glomerular deposits may be of value in classification. For example, predominant IgA (usually associated with IgG) deposits are seen in patients with focal glomerulonephritis and recurrent hematuria with or without proteinuria. The association has been so striking that the term "mesangial IgA nephropathy" has been coined to denote the condition. Circulating IgA-containing immune complexes can be detected in 60% or more of cases of IgA nephropathy, and the rapidity with which hematuria follows an infectious episode suggests that the immune complexes may be formed during antibody excess, possibly with preformed antibody to a common infectious agent of the oropharynx. The large antibody excess complexes would preferentially accumulate in the mesangium. Alternative explanations for the IgA accumulation have included IgA polymers and "antimesangial antibodies." Fifty percent of patients have raised serum IgA levels and evidence of abnormal regulation of IgA production in vitro, suggesting a primary immune abnormality. Occasionally, patients with similar clinical courses have predominantly IgM mesangial deposits.

In systemic lupus erythematosus, the immune complex deposits may be widespread, involving glomeruli and, in 70% of instances, extraglomerular renal tissues as well. Indeed, granular deposits of immunoglobulin and complement in TBM or peritubular capillaries should suggest the diagnosis of SLE. IgA and C1q deposits are prominent in kidneys of patients with SLE—particularly when present diffusely along the GBM—and also suggest this diagnosis.

The identity of the antigen in the glomerular immune complex deposits can be sought either by detection of the antigen with fluoresceinated specific antisera or by elution of antibody from the kidney, with subsequent detection of its reactivity. The immunofluorescence technique may be enhanced by partial elution of the kidney sections with buffers that dissociate immune complexes to uncover antigen sites. Alternatively, the sections can be preincubated with the antigen itself to bind to unoccupied binding sites,

Table 28–5. Generalizations regarding immunofluorescence findings in immune complex and related forms of glomerulonephritis in humans.

Morphologic Type of Glomerulonephritis	Immunofluorescence Findings		
	Granular Immunoglobulin	Granular C3	Fibrinogen-Related Antigen
Proliferative glomerulonephritis			
Diffuse proliferative glomerulonephritis, including poststreptococcal glomerulonephritis	IgG, variable IgA and IgM scattered along the GBM	Similar to immunoglobulin (may be seen when immunoglobulin minimal or absent in poststreptococcal glomerulonephritis)	Usually minimal
Diffuse proliferative, crescent-forming glomerulonephritis	IgG, variable IgA and IgM scattered along the GBM	Similar to immunoglobulin (may be seen when immunoglobulin minimal or absent in poststreptococcal glomerulonephritis)	Heavy in areas of crescents
Focal or segmental proliferative glomerulonephritis	IgG, often prominent IgA, sometimes IgM in a mesangial (or segmental) pattern	Similar to immunoglobulin	Usually absent
Membranous glomerulonephritis	IgG, variable IgA and IgM diffusely along the GBM	Similar to immunoglobulin	Usually minimal
Membranoproliferative glomerulonephritis	IgG, variable IgA and IgM along the GBM in type I; in type II immunoglobulin deposits uncommon	In type I, may overshadow immunoglobulin; in type II, present in the GBM, mesangium, and TBM	Variable
End stage of glomerulonephritis	Variable, IgG, IgA, and IgM, usually most prominent in least damaged glomeruli	Similar to immunoglobulin, may also be present in absence of immunoglobulin	Usually minimal
Systemic diseases with glomerulonephritis (subclassified according to morphologic type)			
Systemic lupus erythematosus	IgG, IgA, and IgM distributed in accord with the morphologic variety; also frequently prominent in the tubulointerstitium	Similar to immunoglobulin	Variable, heavy when crescents present
Henoch-Schönlein purpura	IgG, often prominent IgA, variable IgM may be confined to the mesangium	Similar to immunoglobulin	Frequently prominent

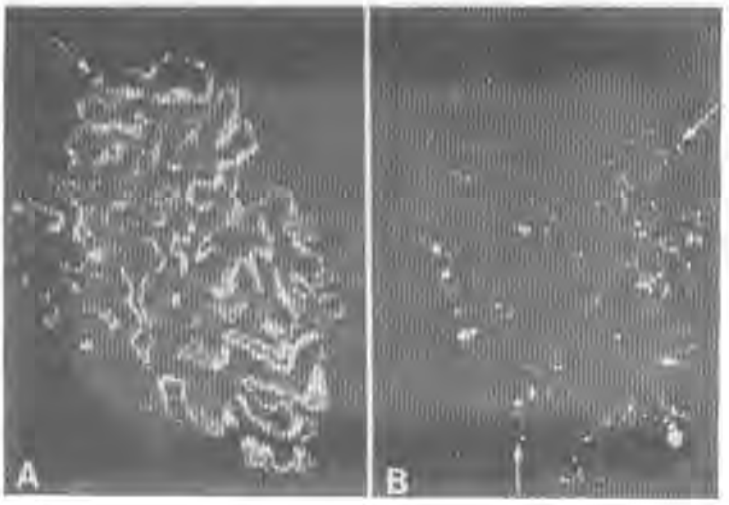

Figure 28–2. Granular deposits of IgG are seen in the glomeruli of patients with immune complex-induced glomerulonephritis. *A:* Heavy diffuse deposits (arrow) are present in a patient with membranous glomerulonephritis and nephrotic syndrome. *B:* Focal granular deposits (arrows), largely confined to the mesangium, are present in a patient with focal proliferative glomerulonephritis and mild proteinuria. (Original magnification × 250.)

increasing the amount and accessibility of the antigen for detection by the fluoresceinated antiserum. Successful elution and recovery of antibody from glomerular immune complex deposits require optimal conditions to recover maximal amounts of functional antibody. Losses can occur through recombination with antigen. Finally, it must be shown that more antibody is present in the eluate than would be expected by simple serum contamination alone.

The patient's physician plays an important part in helping the immunopathologist identify antigen-antibody systems in individuals with immune complex glomerulonephritis. Careful environmental histories may narrow the field of possible antigenic exposures to a few testable systems, with emphasis placed upon detection of chronic, possibly subclinical infective processes. Recent streptococcal infections should be excluded. Serologic testing can be helpful in some cases. Antinuclear and anti-DNA antibodies should be sought, since the nephritis of SLE can occur without other overt organ involvement. Similarly, the presence of rheumatoid factors or cryoglobulins may provide additional insight into the pathogenic process. Antithyroid antibodies, anti-smooth muscle antibody, or other autoantibodies may be an indication of an immune complex system involving endogenous antigens. Screening of sera for antibodies to common viruses or for hepatitis B antigens may be fruitful, particularly when clinical symptomatology suggests viral infection. Abnormalities in serum complement levels (see above) should also be noted.

Several sensitive methods for the detection of circulating immune complexes are now available. Each assay is distinctive in reactivity, sensitivity, and idiosyncrasies, and no single assay is infallible. The use of a combination of assays with different reactivities, therefore, offers the best approach to determining the presence of immune complexes in test sera. Several studies of sera from patients with glomerulonephritis have now been reported. In general, the assays detect large amounts of circulating immune complexes in patients with systemic lupus erythematosus and with glomerulonephritis associated with other systemic immune complex diseases. In primary (presumed immune complex-induced) glomerulonephritis, the frequency of detection is lower than in the systemic disease, and the quantities of immune complexes detected are also less. Immune complexes are present more frequently in acute than chronic glomerulonephritis and in patients with low levels of complement.

The inability of the assays to detect circulating immune complexes in all patients with glomerular immune complex deposits requires careful consideration. Quantitative differences in the load of circulating immune complexes may account for these discrepancies. Thus, large amounts of immune complexes are present in systemic diseases and acute or fulminant glomerulonephritis, and it is possible that the more indolent forms of glomerulonephritis (membranous, membranoproliferative, etc) are mediated by much smaller quantities of circulating immune complexes. The limited sensitivity of the assays could preclude the detection of small quantities. In addition, such patients may have inherent abnormalities causing them to handle more or less physiologic amounts of circulating immune complexes in a nephritogenic manner. Such variations might include the amount and quality of antibody produced or the efficiency of systemic removal of immune complexes from the circula-

tion. The proposed role of low affinity or nonprecipitating antibody in the pathogenesis of membranous forms of glomerulonephritis in patients with systemic lupus erythematosus is a possible example of this concept. Alternatively, circulating immune complexes may be present only intermittently or with changing composition in some forms of glomerulonephritis. The detection of circulating immune complexes would then be influenced by the timing of such determinations. Thus, circulating immune complexes may be present only during the acute phase of poststreptococcal glomerulonephritis or during exacerbations in mesangial IgA nephropathy. Finally, it is possible that the glomerular immune deposits in some forms of glomerulonephritis in fact represent immune complexes formed in situ, and in this situation immune complexes would be present in the glomeruli but not in the circulation. It is clear that serologic immune complex determination cannot yet replace diagnostic renal immunofluorescence. The assays do, however, provide the opportunity for serial studies that may prove to be of clinical and prognostic value and can provide a way of isolating immune complexes for subsequent determination of their antigenic makeup.

Treatment

Nonspecific antiphlogistic and immunosuppressive types of treatment have been widely used, mostly in uncontrolled trials for patients with immune complex-induced glomerulonephritis. The most commonly used agents are corticosteroids, cyclophosphamide, and azathioprine, or occasionally other "immunosuppressive" agents used singly or in combination. The results of these trials are difficult to interpret because of problems with classification, variable treatment regimens, and failure to establish the natural course of the form of immune complex glomerulonephritis under consideration. In general, membranous or membranoproliferative glomerulonephritis does not respond to immunosuppressive treatment, although some investigators believe that corticosteroids may be beneficial. A recent prospective randomized controlled trial demonstrated a beneficial effect of short-term alternate-day prednisone in preserving renal function in membranous nephritis. The true value of this treatment will need to be evaluated in large numbers of patients. Proliferative forms of immune complex glomerulonephritis are usually also unresponsive to immunosuppressive therapy, but occasional apparent success continues to encourage its usage. In contrast, the glomerulonephritis of Wegener's granulomatosis seems to respond favorably to cyclophosphamide. Patients with SLE often show similar benefit from corticosteroid therapy, and the addition of azathioprine treatment may sometimes be appropriate. However, the long-term value of such treatments is still somewhat controversial.

Attempts have also been made to modify the effects of the mediators of immune complex-induced glomerulonephritis, eg, by using antihistamines and antiserotonins. There has been some enthusiasm for anticoagulant and antiplatelet drugs in the management of immune complex and related forms of glomerulonephritis. One carefully controlled trial has shown benefit from the use of low-dose aspirin plus dipyridamole in membranoproliferative (mesangiocapillary) glomerulonephritis. Confirmatory trials and results in other forms of glomerulonephritis are awaited.

Ideally, management of immune complex-induced glomerulonephritis should either eradicate the source of the antigen or inhibit production of the specific antibody. These approaches stress the need for identification of the antigen-antibody systems in each patient. Acute infections, such as streptococcal pharyngitis, should be treated to eradicate the infectious agent. For example, removal of antigen by treating *T pallidum* infection has been beneficial to individuals with syphilitic immune complex glomerulonephritis, as has removal of malignant tissue in immune complex glomerulonephritis associated with neoplasia. The use of plasmapheresis and of specific immunoadsorbents to remove circulating antibody, antigen, or immune complexes is under investigation.

Although in renal transplants recurrence of certain forms of immune complex-mediated glomerulonephritis (membranoproliferative type I, IgA nephropathy, Henoch-Schönlein purpura) is relatively common, significant functional damage to the graft is less common. Recurrence of lupus glomerulonephritis in renal allografts is not as rare as was once thought. Despite the presence of immunosuppression that may prevent recurrent disease in many cases, clinically troublesome lupus glomerulonephritis may recur in the transplant, particularly if the patient has signs of active extrarenal lupus. Because the clinical activity of SLE usually subsides in patients being maintained by chronic dialysis, it is feasible and advisable in most cases to wait until clinical activity has subsided before proceeding to renal transplantation. In addition, focal glomerular sclerosis and membranoproliferative glomerulonephritis type II, diseases in which there is little evidence for immune complex mediation, regularly recur in transplants and cause significant graft loss. Nevertheless, it seems prudent to delay renal transplantation in patients whose renal failure is due to rapidly progressive immune complex glomerulonephritis until the production of nephritogenic immune complexes is minimal.

TUBULOINTERSTITIAL NEPHRITIS

Immune processes that cause injury to the glomerulus also injure extraglomerular renal structures. Just as they do in the glomerulus, anti-basement membrane antibodies and immune complexes can produce tubulointerstitial nephritis. Immunologic tubulointerstitial renal damage can accompany glomerulonephritis or occur as an independent event. Animal models of anti-basement membrane antibody and im-

mune complex-induced tubulointerstitial nepthritis have been developed, and similar processes are beginning to be identified in humans. The role of cell-mediated immunity in tubulointerstitial nephritis is not clearly defined. Evidence exists that sensitized cells may transfer or contribute to tubulointerstitial injury in various experimental animal models, but in humans, although interstitial mononuclear cell infiltration may be conspicuous, the pathogenetic role of cell-mediated immunity other than in renal allografts is not well established.

1. TUBULOINTERSTITIAL INJURY & ANTI-TUBULAR BASEMENT MEMBRANE ANTIBODIES

Major Immunologic Features

- Linear deposits of immunoglobulin, usually accompanied by complement, are found along the TBM.
- Circulating anti-TBM antibodies are often detectable.

General Considerations

The occurrence and pathogenic significance of anti-TBM antibodies has been recognized only recently. Anti-TBM antibodies occur in about 70% of patients with anti-GBM glomerulonephritis. The incidence of these antibodies is perhaps greater in the Goodpasture syndrome type of anti-basement membrane antibody-induced glomerulonephritis, reflecting the broader basement membrane reactivity of the antibody in Goodpasture's syndrome. When present, the anti-TBM antibodies are associated with increased tubulointerstitial injury, indicating their immunopathologic potential. Other situations in which anti-TBM antibodies have occasionally been found are in drug-induced interstitial nephritis, after immune complex-induced glomerulonephritis, and in renal transplants. Rarely, anti-TBM antibodies have been associated with tubulointerstitial nephritis independent of any of the above mentioned conditions.

Immunologic Pathogenesis

The phlogogenic effect of anti-TBM antibodies has been well demonstrated in animal models. Rats or guinea pigs immunized with homologous or heterologous TBM in adjuvant develop anti-TBM antibodies that bind to their TBMs (primarily proximal tubules) and induce interstitial nephritis. Depending on the animal and type of immunization, azotemia, proteinuria, glycosuria, and lysozymuria, singly or in combination, may develop. The importance of antibody in the pathogenesis of the disease is shown by the ability of homologous anti-TBM antibody to transfer the disease in guinea pigs and rats. In Brown-Norway rats immunized with bovine TBM, antibodies form and react with TBM, after which complement becomes fixed and polymorphonuclear leukocytes infiltrate. The polymorphonuclear infiltrate is rapidly replaced by a persisting mononuclear infiltrate, the characteristic cell type in tubulointerstitial nephritis. By the use of monoclonal antibodies directed against cell surface antigens, the infiltrate in the rat was found to contain a significant proportion of helper T lymphocytes with fewer numbers of suppressor T cells and B cells. The presence of multinucleated giant cells and of mononuclear cells that have the ultrastructural characteristics of epithelioid cells indicates that cells of the mononuclear phagocyte system, perhaps acting partially as the accessory effector cells of a cell-mediated type of immune response, also may play an important role in anti-TBM antibody-associated tubulointerstitial nephritis in the rat. In both the guinea pig and rat, anti-TBM antibody-associated tubulointerstitial nephritis has been prevented by the administration of anti-idiotypic antibodies directed against anti-TBM antibodies, thus providing further evidence of the pathogenic importance of the humoral immune response in these 2 animals.

It is of interest that the TBM antigens against which these antibodies react are strain-specific in rats; ie, some inbred strains possess the antigen and others do not. When rats lacking the TBM antigen are given a TBM antigen-positive kidney, they form anti-TBM antibodies reactive with the graft but not with their own kidneys. Such antibodies may contribute to transplant failure in these rats. In humans, similar variability in the occurrence of TBM antigens may account for the fact that anti-TBM antibodies develop in some renal allograft recipients. In other patients, anti-TBM antibodies develop as part of the rejection process. Immune responses to the TBM antigens appear to be genetically influenced. Passive transfer of anti-TBM antibodies in strain XIII guinea pigs has been reported to induce host production of anti-TBM antibodies. To what extent this mechanism operates in humans is unknown.

Anti-TBM antibodies have also arisen in a few patients with methicillin-associated tubulointerstitial nephritis. Experimental studies have shown that native proteins can be made immunogenic to a host animal by conjugation with a foreign hapten. Methicillin derivatives (and those of other penicillin analogs) can form stable conjugates with proteins, and it has been demonstrated that methicillin derivatives can bind to the TBM. The drug-TBM conjugate may induce a humoral immune response with subsequent development of anti-TBM antibodies. Since anti-TBM antibodies are an unusual finding in drug-induced tubulointerstitial nephritis, other mechanisms such as unidentified cellular immune responses may play a role in the tubulointerstitial injury.

Anti-TBM antibodies have been found in occasional patients with immune complex-induced glomerulonephritis, particularly children. In some of these patients, the immune complex glomerular injury preceded the formation of anti-TBM antibodies, suggesting that immunologic damage to the kidney may on rare occasions trigger subsequent autoantibody formation. Anti-TBM antibodies have also been iden-

tified in a few children with suspected primary tubulointerstitial nephritis.

Clinical Features

When anti-TBM antibodies occur with anti-GBM or immune complex-induced glomerulonephritis, the clinical course is generally that of the underlying glomerulonephritis. In transplant recipients it is difficult to distinguish the effects of anti-TBM antibodies from the rejection process itself. Since anti-TBM antibodies can induce severe tubulointerstitial nephritis in animals, one can hardly discount their importance in patients with glomerulonephritis or transplant rejection. Some patients with anti-TBM antibody-induced tubulointerstitial nephritis may manifest complete or partial Fanconi's syndrome.

Immunologic Diagnosis

Diagnosis of anti-TBM antibody-associated tubulointerstitial nephritis is based on the detection by immunofluorescence of linear deposits of immunoglobulin and complement along the TBM (Fig 28–3A). As in anti-GBM antibody-disease, the specificity of the reaction should be confirmed by elution studies or by detection of circulating anti-TBM antibodies. Serum is usually tested by indirect immunofluorescence on normal kidney targets for the presence of circulating anti-TBM antibodies. Radioimmunoassays are also available.

Treatment

The associated glomerulonephritis is treated as outlined in previous sections.

2. TUBULOINTERSTITIAL INJURY & IMMUNE COMPLEXES

Major Immunologic Features

- Immunoglobulin and complement are present in a granular pattern along the TBM, in the interstitium, or in the walls of peritubular capillaries.
- Circulating immune complexes may be detected.

General Considerations

In some patients, the tubulointerstitial damage associated with glomerulonephritis may be due in part to extraglomerular renal deposition of immune complexes. Granular deposits of immunoglobulin and complement are seen along the TBM or peritubular capillaries or in the interstitium of 50–70% of patients with lupus nephritis. The deposits' presence generally correlates positively with the severity of the tubulointerstitial histopathology. Extraglomerular deposits have also been found, though with much lower frequency, in cases of cryoglobulinemia and in membranoproliferative and rapidly progressive glomerulonephritis, Sjögren's syndrome, and primary idiopathic interstitial nephritis. Prominent immune complex localization in extraglomerular renal sites in the absence of glomerular immune complex deposits is uncommon. A few patients with SLE have been noted to have predominant or exclusive localization in the interstitium.

Immunologic Pathogenesis

The role of interstitial immune complex deposition

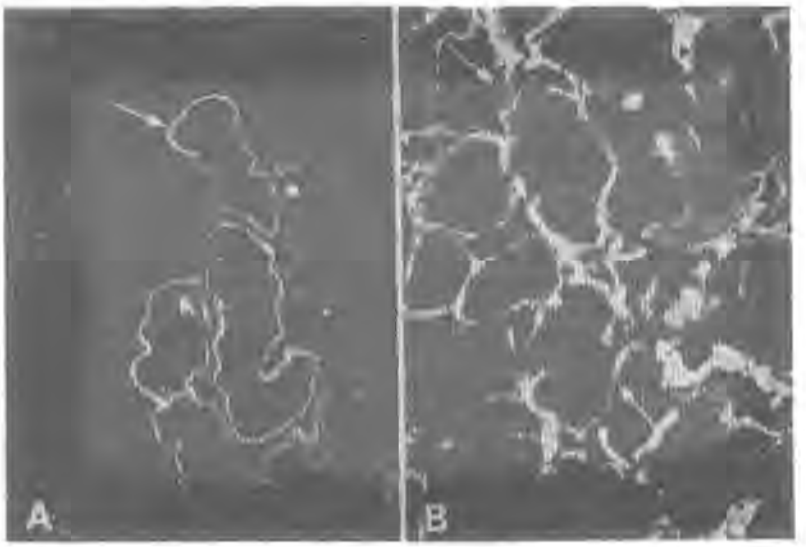

Figure 28–3. *A:* Linear deposits of IgG (arrow) are present along the TBM of focal renal tubules in the renal biopsy of a patient with anti-GBM glomerulonephritis. *B:* Diffuse granular deposits of IgG (arrows) are seen along the TBM of most renal tubules in the renal biopsy of a patient with systemic lupus erythematosus and immune complex glomerulonephritis. (Original magnification × 250.)

in inducing tubulointerstitial injury has been determined by studying chronic serum sickness in rabbits. Rabbits having active immune responses and forming large amounts of immune complexes accumulate these complexes on TBM, in peritubular capillaries, and in interstitial spaces in addition to the GBM. Along with such deposits, these rabbits have prominent tubulointerstitial histopathologic injury that is much more severe than that in rabbits with glomerular immune complex deposition alone. Extraglomerular renal deposits of immune complexes and consequent interstitial damage are frequent in SLE (Fig 28–3B). In some patients with SLE (just as in some rabbits with chronic serum sickness), circulating immune complexes may be unique in quantity—or quality—which may allow these complexes to become deposited in the renal interstitium in addition to the glomerulus.

In experimental animals, immune complexes may form locally in the renal interstitium. Rabbits given repeated injections of a soluble basement membrane-poor extract of renal cortex develop tubulointerstitial lesions and sometimes glycosuria and aminoaciduria. Immunofluorescence studies reveal granular deposition of IgG and C3 along proximal TBMs. Sera from the injected rabbits contain antibodies reactive with antigens normally present in the cytoplasm of proximal tubular cells. It is postulated that the deposits along the TBM result when circulating autoantibodies complex with autologous antigens as they move out of tubular cells. In another model, rats immunized with urinary Tamm-Horsfall glycoprotein develop immune deposits along the ascending thick limbs of the loop of Henle, macula densa, and portions of the distal tubule, sites of production of the protein. Only mild interstitial infiltration of mononuclear cells occurs, but these models demonstrate that in situ tubulointerstitial immune complex formation has a potential for injury in humans.

Clinical Features

The clinical course is usually that of the associated glomerulonephritis. Evidence of tubular dysfunction may be present.

Immunologic Diagnosis

The tubules, vessels, and interstitium should be examined with particular care during study of renal biopsies by immunofluorescence. Interstitial deposits of immunoglobulin and complement are usually focal and less intense than glomerular deposits and may easily be overlooked. Prominent or widespread tubulointerstitial deposits suggest systemic lupus erythematosus.

Treatment

The underlying glomerulonephritis is treated as outlined in previous sections.

3. TUBULOINTERSTITIAL INJURY & CELL-MEDIATED IMMUNITY

Major Immunologic Features

- Extensive mononuclear cell interstitial infiltrates; recent studies suggest the presence of T lymphocytes.
- No detectable antibody or immune complexes along TBM or in the circulation.

General Considerations

Interstitial mononuclear cell infiltrates without detectable anti-TBM antibody or immune complexes are the most common form of tubulointerstitial nephritis in humans. Frequently the disease appears to be a hypersensitivity reaction to drugs, which may include a wide range of antibiotic, nonsteroidal anti-inflammatory, diuretic, and other drugs. T lymphocytes can be demonstrated in the extensive mononuclear cell infiltrates in renal biopsies, and in some cases tests of delayed hypersensitivity show sensitivity to the drug in question, suggesting a cell-mediated immune reaction. Unfortunately, no satisfactory animal model of drug-induced tubulointerstitial nephritis exists for a further examination of immunologic mechanisms.

Acute renal allograft rejection in humans clearly involves cell-mediated immune reactions concerned with histocompatibility antigens, and the tubulointerstitial nephritis associated with anti-GBM nephritis, pyelonephritis, sarcoidosis, and idiopathic interstitial nephritis may also involve cellular immune mechanisms. Some patients with Sjögren's syndrome have peritubular interstitial lymphocyte infiltrates and renal tubular acidosis, particularly distal type I. In addition, renal allograft rejection and hypergammaglobulinemic states such as chronic active hepatitis and essential cryoglobulinemia may also be associated with renal tubular acidosis. It seems likely that some form of autoimmunity to renal tubular cells is present in these patients.

Immunologic Pathogenesis

The role of cell-mediated immunity in the pathogenesis of tubulointerstitial nephritis is less well defined than the role of the humoral immune response. However, the presence of lymphocytes in human tubulointerstitial nephritis as well as in experimental animal models of the disease suggests a role for the cell-mediated immune response. It has recently been reported that tubulointerstitial nephritis is transferable by immune cell suspensions, but not by serum, in both rat and murine models. Experimental evidence suggests that even in anti-TBM antibody-associated nephritis in the guinea pig and rat, sensitized cells are present that may contribute to the progression of disease, even if they are not themselves capable of initiating the full spectrum of disease after passive transfer. In Brown-Norway rats with anti-TBM antibody-associated nephritis, for example, sensitized immune cells induce a mild, but definite, lesion following subcapsu-

lar renal transfer to the kidneys of naive recipients. Lewis rats develop a nodular granulomatous form of tubulointerstitial nephritis when immunized with Brown-Norway rat renal basement membrane. The lesion is easily transferable with intravenous administration of immune lymph node cells. Murine anti-TBM antibody-associated tubulointerstitial nephritis also is transferable with cells that have been grown in tissue culture. The lesion develops slowly, and generation of effector cells is suggested. Guinea pigs with anti-TBM-associated nephritis show both in vivo delayed hypersensitivity to soluble renal tubular antigens and in vitro correlates of the delayed hypersensitivity reaction in response to the same antigens. Immune cells from guinea pigs with anti-TBM antibody-associated nephritis have been shown to home specifically to the kidneys of naive recipients, to be cytotoxic in vitro for target kidney cell monolayers, and to partake in antibody-dependent cellular cytotoxicity reactions in vitro. T lymphocytes from guinea pigs secrete a factor that has been held responsible for mediating fibroblast proliferation and collagen synthesis, thus providing one potential explanation for the interstitial fibrosis observed in cases of chronic interstitial nephritis.

Experimentally induced delayed hypersensitivity can be produced in renal structures by exogenous antigens. The best-characterized model is that induced by transfer of cells sensitized to bovine γ-globulin into rats that receive aggregated bovine γ-globulin introduced in their renal cortex. A mononuclear cell infiltrate and injury ensue. The role of cell-mediated immunity in both human and experimental tubulointerstitial nephritis remains to be fully elucidated, but as is also the case in the study of glomerulonephritis, a growing appreciation is developing for the potential role of sensitized cells in the pathogenesis of tubulointerstitial nephritis.

Clinical Features

Drug-induced acute tubulointerstitial nephritis presents a typical acute clinical picture of fever, rash, and hematuria, with a rising serum creatinine level and eosinophilia following a course of drug therapy. Other features such a pyuria and eosinophiluria, mild proteinuria, flank pain, and arthralgia may also be present, together with an elevated serum IgE concentration. Progressive renal failure is the general rule until the offending drug is discontinued. Acute renal allograft rejection is characterized by fever, a rising creatinine level, and graft swelling, with decreasing urine output. The clinical features of idiopathic tubulointerstitial nephritis are similar to the drug-induced form of interstitial nephritis.

Immunologic Diagnosis

Examination of renal biopsy material confirms extensive mononuclear cell infiltrates, often including eosinophils in drug-induced tubulointerstitial nephritis. Recently T cells have been identified using monoclonal antibodies. Immunofluorescence usually shows an absence of linear deposits of immunoglobulin along the TBM; however, in drug-induced cases renal biopsy specimens can be tested for drug deposition along basement membranes using specific fluoresceinated antiserum to the drug. The patient's reactivity to the drug can be tested by hemagglutination assay to detect antibodies and by in vitro assays of cellular sensitivity performed with peripheral blood lymphocytes. As monoclonal antibodies are applied to the study of T cell subsets and macrophages, better characterization of the different types of cell-mediated tubulointerstitial nephritis should evolve.

Treatment

In cases of drug-induced interstitial nephritis, the offending drug should be immediately discontinued and replaced as needed by a suitable structurally unrelated alternative drug. Corticosteroids may aid in quicker resolution of drug-induced tubulointerstitial nephritis and are valuable in acute renal allograft rejection. T cell monoclonal antibodies have also been used successfully in treating renal transplant rejection.

OTHER GLOMERULAR DISEASES OF SUSPECTED OR UNCERTAIN IMMUNE PATHOGENESIS

In addition to those nephritides already discussed, which clearly are due to immunologic disorders, there is a group of glomerular diseases in which the role of immune mechanisms is uncertain. These diseases include minimal change nephropathy, focal glomerulosclerosis, vasculitides, diabetes mellitus, some disorders of coagulation, amyloidosis, and plasma cell dyscrasias. With the exception of minimal change nephropathy and diabetes mellitus, these disorders account for only a small percentage of cases of glomerular injury.

Minimal Change Nephropathy

Minimal change nephropathy is the most common cause of the nephrotic syndrome in children and also occurs, though less commonly, in adults. The glomerular lesion is characterized by minimal or no histologic changes at the light microscopic level, no immune reactants demonstrable by immunofluorescence microscopy, but diffuse effacement of the epithelial cell foot processes observed on ultrastructural examination. Typically, these patients have selective proteinuria and a good response to corticosteroid or cyclophosphamide therapy. This condition is not thought to be mediated by immune complexes or anti-kidney antibodies; however, a few investigators have been able to demonstrate circulating complexes in the sera of such patients. There remains a large number of interesting observations that might suggest a possible immunologic origin of minimal change nephropathy. Lymphocytotoxic antibodies have been demonstrated in the sera of minimal change patients, as have high levels of immunoconglutinin, especially during relapse. High levels of circulating IgM and IgE and depressed levels of IgG and IgA have been noted,

and a relative lymphocytopenia is a frequent observation. Although a few children have been reported who get seasonal exacerbations of nephrotic syndrome in association with allergic asthma, claims for a pathogenic role for IgE-containing immune complexes have been dismissed in further studies. Another association is with nonsteroidal anti-inflammatory drugs, which, in addition to occasionally causing interstitial nephritis or changes in intrarenal hemodynamics, have also in a few patients reportedly caused nephrotic syndrome with minimal change histologic features. However, a role for prostaglandins in the susceptibility to the idiopathic form of minimal change nephropathy has not been demonstrated.

A number of findings suggest a possible cell-mediated mechanism of injury. Remission can be induced by an intercurrent measles infection, which is known to be associated with depressed cell-mediated immunity. A minimal change lesion is sometimes seen in Hodgkin's disease in adults, and lymphocytes from minimal change nephropathy patients can be shown to have been sensitized to fetal kidney antigens in migration inhibition experiments. A blastogenesis-inhibiting factor has been described in the sera of minimal change patients. It is possible that some cellular immune mechanism is instrumental in producing glomerular protein leakage in minimal change nephropathy, possibly through defective T cell regulation, leading to the production of a soluble factor that results in increased glomerular permeability.

Focal Glomerulosclerosis

Focal glomerulosclerosis is another common cause of nephrotic syndrome in children and young adults. The condition is usually steroid-resistant and usually progresses slowly but relentlessly to end-stage renal failure. The histologic change consists of hyalinosis and sclerosis, which begins focally and also segmentally within individual glomeruli. Immunofluorescence microscopy shows coarse granular accumulations of IgM, often with C3 and fibrin, within the areas of sclerosis. It has been suggested that these findings are due to nonimmunologic trapping in areas of damaged mesangium, and an "immune complex" pathogenesis is not strongly suspected. Assays for circulating immune complexes are positive in a minority of patients. No consistent abnormality of circulating immunoglobulins, complement, or cellular immunity has been identified. It has been held that the disease commences in juxtamedullary glomeruli, permitting needle biopsies of the kidneys sometimes to miss the affected tissue. This can cause confusion with minimal change nephropathy, though a close or even overlapping etiologic relationship has also been suggested. An experimental model of focal glomerulosclerosis has been reported in rats repeatedly subjected to the nephrotoxic effects of the aminonucleoside of puromycin. No effective treatment of focal glomerulosclerosis has yet been discovered, although several trials of steroids, immunosuppression, antiplatelet agents, and plasmapheresis have been conducted. Focal glomerulosclerosis is usually an idiopathic disease; however, an apparently identical disease may develop in heroin abusers and some patients with AIDS.

Vasculitis

The vasculitides are made up of syndromes with a spectrum of clinicopathologic features with the essential common component of vasculitis, an inflammatory reaction in vessel walls, leading to ischemia of the supplied tissues. Increasing evidence points to an immunologic pathogenesis of the vascular lesions—in particular, the deposition of circulating immune complexes triggering the inflammatory process. The mediation of injury is brought about by the action of neutrophils, complement, and mononuclear cells as well as platelets and vasoactive amines. Cellular immunity is also involved, and true granulomatous reactions may be observed in some of these conditions. The vasculitides are classified according to several different features, including the type and size of the vessels affected, the involvement of specific organs such as skin, lung, or kidneys, the characteristics of the inflammatory reaction—in particular granuloma formation—and the clinical features. Utilizing pathologic, clinical, and immunopathogenic criteria, several distinct disorders can be identified. Several of the vasculitides commonly involve the renal vessels.

Polyarteritis nodosa is characterized by a necrotizing vasculitis of small and medium-sized muscular arteries. Aneurysmal dilatations may be produced, and the lesions are usually segmental. Hepatitis B antigenemia has been detected in up to 50% of cases, occasionally with immunofluorescence evidence of vascular deposition of hepatitis B antigen and antibody in vessel walls. Widespread involvement of vessels leads to diffuse organ involvement, with the kidney being frequently affected. Renal injury is primarily brought about by vasculitis of the arcuate and interlobular vessels, with resulting ischemic glomerular changes.

The syndromes collected under the heading of hypersensitivity vasculitis have the common feature of involvement of smaller vessels, in contrast to those affected in the polyarteritis nodosa groups. Typically, the skin is involved with a characteristic leukocytoclastic vasculitis involving postcapillary venules. Endogenous and exogenous antigens are being associated with the syndromes with increasing frequency, and immune complex deposition has been shown to trigger the vasculitis in many cases. The syndromes commonly involving the kidney include serum sickness, Henoch-Schönlein purpura, and essential mixed cryoglobulinemia. Henoch-Schönlein purpura may be associated with preceding infections (often respiratory) and food and drug allergies. Abdominal pain, fever, malaise, arthralgia, edema, and a characteristic nonthrombocytopenic purpuric skin rash are seen. Glomerulonephritis occurs in a significant proportion of patients. Diffuse mesangial IgA, C3, and fibrin are found on immunofluorescent examination of biopsy tissue. The syndrome usually occurs in children and follows a relapsing course, but with progression of re-

nal disease in a minority of patients. Essential mixed cryoglobulinemia is produced by the deposition of immune complexes made up of monoclonal IgM, rheumatoid factor, and polyclonal IgG. Purpura, arthralgia, fever, and hepatosplenomegaly are the typical clinical features. A rapidly progressive glomerulonephritis with diffuse endocapillary proliferation associated with endomembranous deposits and crescents can occur. IgG, IgM, and C3 can be demonstrated in the glomeruli by immunofluorescence microscopy. Hepatitis B antigen has also been found in association with this syndrome.

Wegener's granulomatosis is a syndrome comprised of granulomatous vasculitis involving the upper and lower respiratory tracts, disseminated small vessel vasculitis, and glomerulonephritis. The renal lesion consists of a segmental necrotizing glomerulonephritis with associated granulomatous vasculitis. The underlying antigens remain unidentified, but circulating immune complexes have been detected in some patients. Immune reactants including IgG and complement have been inconsistently seen in the glomeruli of renal biopsy specimens and electron-dense deposits occasionally seen on electron microscopy. This vasculitis is particularly responsive to cyclophosphamide therapy.

Diabetes Mellitus

Although the renal involvement in diabetes is variable, a large number of patients develop proteinuria within 20 years after the onset of diabetes and frequently die from renal failure within 5 years. It is still controversial whether good control of blood glucose levels can prevent this progression. The primary renal insult is microangiopathic. There is little evidence that immunologic mechanisms contribute to renal impairment of persons with diabetes. The striking linear accumulations of IgG, albumin, and other major plasma proteins found in the basement membranes of diabetics (both glomerular and tubular) are not thought to have immunologic specificity. They recur in kidneys that are transplanted from nondiabetic donors to diabetic recipients, thus implicating the hyperglycemic milieu of the diabetic rather than the genetic constitution of the basement membranes. Experimental diabetes induced by alloxan or streptozocin in rodents leads to accumulation of IgG and C3 in the mesangium of glomeruli, not in the glomerular basement membranes. However, chemically induced diabetes in rhesus monkeys leads to the same immunofluorescence microscopic findings as in humans. Thus, the site of accumulation of immunoglobulins and other proteins within the structures of the diabetic glomerulus appear to relate to differences between various suborders of mammals rather than to different etiologic causes of diabetes. Another phenomenon occasionally observed in diabetes is the glomerular trapping of circulating beef insulin antigen-antibody immune complexes in patients receiving beef insulin injections. This is most probably merely an interesting epiphenomenon and not of great immunopathologic importance.

Coagulopathies

Several systemic coagulation syndromes can cause acute renal failure, including hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, and postpartum renal failure. Disseminated intravascular coagulation may occur as a secondary if not the primary event in all 3 syndromes. The lesions have certain similarities to those seen in immunologically induced hyperacute rejection in renal allograft recipients, a lesion that has striking glomerular fibrin accumulation. In the 3 syndromes mentioned here, infectious agents (or a retained product of conception) may act, perhaps through immunologic mechanisms, to cause intravascular platelet aggregation as the triggering event. Alterations in levels of arachidonic acid derivatives, especially prostacyclin and thromboxane, have been suggested recently to be responsible for pathologic platelet aggregation in these coagulopathies. Low levels of prostacyclin have been found in adults and children with hemolytic-uremic syndrome and in some unaffected relatives. Therapeutic trials using prostacyclin, fresh-frozen plasma and plasmapheresis in these diseases are underway. When immunoglobulin and complement deposits accompany the glomerular fibrin deposits, an immunologic basis for the coagulopathy is suggested.

A "localized" coagulopathy, renal vein thrombosis, can also affect the kidney. This diagnosis is suggested when back pain is associated with the nephrotic syndrome and can be confirmed by renal venography. Renal vein thrombosis is usually seen in association with the nephrotic syndrome and glomerulonephritis, with heavy granular IgG and C3 deposits. The diagnosis is suggested by observation of margination of polymorphonuclear leukocytes in the glomerular capillary. The relationship between glomerulonephritis and renal vein thrombosis has been elusive and controversial. Currently, it is thought that most instances of thrombosis are secondary to the underlying glomerular disease; however, the suspicion remains that in isolated cases primary renal vein thrombosis might induce renal injury, releasing renal antigens and thereby initiating a nephritogenic immune reation.

Amyloidosis & Plasma Cell Dyscrasias

Renal amyloid deposition may occur in association with plasma cell dyscrasias and may also be seen in conditions characterized by chronic or recurrent infection or inflammation. Examples are rheumatoid arthritis or familial Mediterranean fever. Clinical features include proteinuria, the nephrotic syndrome, and renal failure. Underlying systemic disease, particularly multiple myeloma, should be sought when a diagnosis of amyloidosis is confirmed. Renal amyloidosis is usually diagnosed by demonstrating abnormal accumulations of proteins in a renal biopsy with special histochemical stains. In primary amyloidosis, which has an associated plasma cell proliferation and often multiple myeloma, the deposited protein has features of immunoglobulin light chains. Immunofluorescence studies with light chain-specific

reagents may be a useful diagnostic approach. In amyloidosis secondary to chronic inflammation, amyloid protein A is identified in the deposits. This protein is related to a serum protein that acts as an acute phase reactant. Another component of amyloid, the P component, is quite similar to C-reactive protein. This P component has recently been shown to be a constituent of normal human GBM. Additional types of amyloid protein are being recognized, and it is anticipated that further definition of this group of diseases may develop on a biochemical basis.

Acute renal failure is often associated with multiple myeloma (the so-called myeloma kidney). Many different factors are present to induce renal damage. They include hypercalcemia, hyperuricemia, and fluid depletion, as well as the toxic effects of Bence Jones proteins (immunoglobulin light chains) found inspissated within the lumens of the tubules. In addition, diffuse renal deposition of amyloid proteins may produce chronic renal injury. A form of nodular glomerulopathy has recently been described in which kappa light chains accumulate segmentally within glomerular mesangial regions, causing rapid impairment of renal function; this disorder appears to respond well to plasmapheresis therapy. Monoclonal IgG cryoimmunoglobulins may be found in multiple myeloma and may be deposited within glomeruli. Cryoimmunoglobulinemia is more commonly found in Waldenström's macroglobulinemia (plasma cell dyscrasia producing IgM). Uncommonly, glomerular disease can be produced acutely by the deposition of occlusive thrombi of IgM. However, renal involvement in macroglobulinemia occurs less frequently than renal disease in myeloma.

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29 Dermatologic Diseases

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Advances in immunologic laboratory methods and a better understanding of the basic immunologic mechanisms of tissue injury, including autoantibody formation, immune complexes, and cell-mediated immunity, are making valuable contributions to research in the pathogenesis of skin disease. Immunofluorescence techniques have demonstrated the deposition of various immunoglobulins, complement components, and alternative pathway components of complement activation at the site of lesions in various bullous diseases, including dermatitis herpetiformis and bullous pemphigoid. In addition, direct immunofluorescence techniques have demonstrated the presence of immunoglobulins and complement, presumably in the form of immune complexes, in the blood vessel walls of patients with various forms of vasculitis. Immunoelectronmicroscopic studies have provided important new information suggesting the existence of epidermolysis bullosa acquisita and IgA bullous dermatosis as distinct entities. At present, immunologic data also indicate an important immunopathologic role of cell-mediated immunity in allergic contact dermatitis and photoallergic contact dermatitis. Preliminary data suggest an important role of normal host cell-mediated immunity in the defense against cutaneous fungi. Absence of this cell-mediated immunity may result in widespread cutaneous fungal diseases as well as mucocutaneous candidiasis.

ALLERGIC CONTACT DERMATITIS

Major Immunologic Features

- T cell-mediated eczematous disease.
- Characterized by a 48-hour delayed eczematous response to the epicutaneous application of the allergen.

General Considerations

Allergic contact dermatitis is an example of a disease involving cell-mediated immunity. Although the exact incidence of allergic contact dermatitis in the general population is unknown, it is certainly the most common immunologic disease encountered by dermatologists. In fact, about 3–5% of patients seen by dermatologists are evaluated for possible allergic contact dermatitis. The potential contact sensitizing antigens to which humans are exposed are multitudinous and include drugs, dyes, plant oleoresins, preservatives and metals. The 5 most common contact sensitizing antigens encountered in clinical practice are *Rhus* species of plants (poison ivy, oak, or sumac), paraphenylenediamine, nickel compounds, rubber compounds, and the dichromates.

Immunologic Pathogenesis

The exact underlying immunologic basis of allergic contact dermatitis is unknown. Much has been learned by experimental application of simple chemicals such as dinitrochlorobenzene (DNCB) to the skin of guinea pigs. It is hypothesized that these chemicals react with skin components to form hapten-carrier molecules. The precise identification of the hapten-carrier molecule responsible for sensitization has been difficult, since DNCB is a highly reactive substance that can form dinitrophenyl-protein bonds with a variety of tissue substances. Early experiments showed that induction of sensitization to a topically applied antigen required an intact local lymphatic system and regional lymph node. Severing the local lymphatics or excising the regional lymph node prevented active cutaneous sensitization but did not interfere with a secondary response once sensitization was established. The participation of regional lymph nodes was demonstrated by the observation of their proliferation (predominantly in the thymus-dependent paracortical area) following the experimental induction of allergic contact dermatitis in animals.

The primary role of the regional lymph node and lymphatic system has been made doubtful by the suggestion that the "training ground" for actively sensitizing the lymphocytes occurs at the site of antigen deposition. When radiolabeled antigen was injected intradermally, much of this antigen rapidly disappeared from the injection site. Subsequently, the antigen could not be identified in the local lymphatic system or regional lymph node. However, a small quantity of antigen remained at the site of injection, and the intensity of the cell-mediated response could be directly correlated with the persistence of this local antigen depot. These data suggest that peripheral sensitization at the site of antigen deposition can occur and that regional lymph node proliferation may occur as sensitized lymphocytes recruit other lymphocytes. It is not known whether these 2 sets of experiments, one demonstrating peripheral sensitization and the other the need for an intact regional lymph node and lymphatic system in the development of allergic con-

tact dermatitis, are contradictory or represent a difference in experimental design.

The dendritic Langerhans cell, which makes up 2–8% of the epidermal cell population, is a bone marrow-derived macrophage expressing Ia cell surface antigens and Fc and C3 cell surface receptors. The Langerhans cell is capable of binding and presenting antigens to sensitized T cells and participating in allogeneic T cell stimulation. There is also good evidence suggesting that prior exposure of epidermal cells to ultraviolet light results in (1) depletion of ATPase-positive cells (presumably Langerhans cells) and (2) interference with the function of the remaining Langerhans cells. Such treatment blocks optimal sensitization to epicutaneously applied dinitrofluorobenzene and is also capable of inducing antigen-specific unresponsiveness (tolerance). One recent study indicates that the tolerance is due at least in part to the generation of suppressor T cells.

These data relating to Langerhans cells suggest that the immune system at the most peripheral region of the body (ie, the epidermis) has an intact antigen-processing macrophage system capable of providing a "training ground" for the active sensitization of lymphocytes. Furthermore, these studies indicate that exposure to ultraviolet light can profoundly impair the host's ability to mount a local as well as systemic T cell response.

In addition to the immunologic functions of the Langerhans cells, there is also evidence that the resting epidermal keratinocyte may play a significant role in the development of a localized and possibly a systemic T cell response. These keratinocytes synthesize and secrete a 15,000-MW substance possessing interleukin-1 (IL-1) activity. This factor, like IL-1, when cultured in vitro with thymocytes, induces the thymocytes to undergo a proliferative response; thus, it has been termed epidermal thymocyte-activating factor (ETAF). In addition, ETAF promotes the release of interleukin-2 (IL-2). These studies suggest that the epidermal keratinocyte may secrete a number of factors that promote maturation of T cells.

One additional fascinating aspect of experimental allergic contact dermatitis is that by prior oral feeding of DNCB one can induce an immunologic specific unresponsiveness to epicutaneous sensitization with DNCB. Recent studies suggest preferential stimulation of suppressor T cells by oral ingestion of antigen as the mechanism for this unresponsiveness. The significance of this intriguing and readily reproducible phenomenon, initially reported by Sulzberger and Chase, is unknown.

The central role of the lymphocyte in the pathogenesis of allergic contact dermatitis was first reported in the classic experiment of Landsteiner and Chase in 1942. These investigators specifically transferred allergic contact dermatitis from a sensitized guinea pig to an unsensitized one by the intraperitoneal injection of peritoneal exudate cells. Similar experiments substituting serum for peritoneal exudate cells failed. Furthermore, lymph node lymphocytes from sensitized animals, placed in tissue culture with the appropriate sensitizing antigen, underwent a proliferative response measured by increased DNA synthesis. In mice, the proliferative response of sensitized lymphocytes to the antigen could be abolished by pretreatment of the lymphocytes with anti-Thy-1 (θ) serum, which suggested that T lymphocytes are involved in the sensitization process.

In addition to these animal studies, in vitro studies in humans have demonstrated that peripheral lymphocytes from some patients with allergic contact dermatitis, cultured with the sensitizing antigen, undergo a proliferative response. In fact, peripheral lymphocytes from a patient suffering from nickel contact dermatitis produced migration inhibitory factor (MIF) on exposure to nickel in tissue culture.

Several years ago, Polak et al presented some evidence to indicate that the spontaneous flare of chromium dermatitis may involve plasma cells. Their experiments in animals failed to detect circulating antibody to chromium. Furthermore, it was possible to specifically transfer this "flare-up" reaction by the intradermal injection of peritoneal exudate cells from sensitized donors into normal recipients followed by the intravenous injection of chromium. However, peritoneal cells from animals permanently desensitized (tolerant) to contact hypersensitivity to chromium continued to produce "flare reactions" when injected into normal animals. These data suggest that small quantities of chromium may be absorbed from a site of inadvertent contact and could be systemically transported to sites of previous eczematous disease. There, the antigen would activate previously sensitized lymphocytes, producing reexacerbation of the eczematous disease.

Clinical Features

Allergic contact dermatitis is an eczematous reaction. Eczema is characterized in the acute form by erythema, edema, and vesiculation and in the chronic form by scaling. Histologically, eczematous lesions are characterized by a perivascular mononuclear infiltrate and varying degrees of dermal and epidermal edema (spongiosis).

The site of allergic contact dermatitis is often a clue to diagnosis. For example, nickel contact sensitization may be manifested by an eczematous reaction on the ear lobes, around the neck, and on the upper mid back, the wrists, and upper thighs. These sites correspond to points of contact with earrings, necklaces, brassiere clasps, bracelets or wrist bands, and girdle clasps, respectively. Paraphenylenediamine sensitivity occurs in individuals using hair dyes or sunscreens that contain PABA, which cross-reacts. Sensitivity to rubber results from chemical additives or antioxidants. Dichromate reactivity occurs in leather tanning workers or wearers of leather.

Immunologic Diagnosis

The diagnosis of allergic contact dermatitis is based on the distribution of the lesions, an exhaustive his-

tory, and examination of the patient's home and place of work for possible sensitizing compounds. The diagnosis is confirmed by patch testing, a procedure introduced in 1896 by Jadassohn. Patch testing consists of applying a nonirritating (low) concentration of the suspected contact antigen to the patient's skin, usually the back, and covering with an occlusive dressing. The dressing is removed after 48 hours. An eczematous reaction at the site of the patch test constitutes a positive response.

Differential Diagnosis

An eczematous reaction is not pathognomonic of allergic contact dermatitis. Similar skin reactions occur with bacterial and fungal diseases (infectious eczematoid dermatitis). Eczema also occurs when the skin is repeatedly traumatized by scratching (neurodermatitis) or following the cutaneous application of harsh chemicals and solvents (primary irritant dermatitis). Eczematous lesions are characteristic features of certain inherited diseases such as atopic dermatitis, X-linked hypogammaglobulinemia (see Chapter 20), and Wiskott-Aldrich syndrome (thrombocytopenia, recurrent infections, and eczema). These conditions can be differentiated from allergic contact dermatitis on the basis of the history and clinical features and by patch testing (see above).

Treatment

Avoidance of exposure to an identified allergen is theoretically curative, but avoiding known allergens may prove difficult. For example, common sensitizers such as benzocaine are employed in a variety of topical medications such as sunburn preparations and antiseptic creams. If individuals with sensitivity to benzocaine fail to read the labels on these preparations, they may inadvertently reexpose themselves to the offending agent. Another example of unwitting exposure to a known allergen such as poison ivy is contact with the smoke from burning leaves. If poison ivy is present in the leaves, the pentadecacatechols, the allergen in poison ivy, will vaporize and can be transmitted to a sensitized individual as an aerosol without direct contact with the plant.

In addition, the patient may exacerbate an allergic contact dermatitis by exposure to cross-reacting chemical compounds that are quite similar to the allergen to which the patient was originally sensitized. The most common example of a cross-reacting chemical is the para-aminobenzoic acid group (PABA). PABA is used in sunscreens and will cross-react with sulfonamides, sulfonylurea diuretics (eg, chlorothiazides), azo dyes, and topical analgesics (eg, benzocaine).

One valuable clinical tool in the management of persistent allergic contact dermatitis is to have patients bring in the contents of their medicine cabinets. This simple procedure will often lead to prompt identification of the offending agent.

Symptomatic treatment consists of the application of wet dressings employing Burow's solution and a 1% hydrocortisone cream or lotion. Oral or parenteral corticosteroids (eg, triamcinolone, 40 mg intramuscularly per week, or prednisone, 40 mg orally daily) may be needed temporarily in severe cases.

Oral desensitization with poison ivy extracts has been employed with some claims of success. Although data are now available that indicate that one can produce tolerance to DNCB by oral administration, the clinical feasibility of oral desensitization remains unproved.

Prognosis

Most patients with allergic contact dermatitis respond to the procedures mentioned above. In some cases—particularly chromium contact dermatitis—the course is chronic with frequent spontaneous exacerbations.

PHOTOALLERGIC CONTACT DERMATITIS

Major Immunologic Features

- T cell-mediated eczematous disease.
- Can be passively transferred by cells.
- Ultraviolet light plays an essential role in generating the allergen.

General Considerations

Photoallergic contact dermatitis is characterized by a chronic eczematous reaction in areas of skin exposed to light (the backs of the hands, the face, the V of the neck). Unexposed areas of skin are usually not affected.

Immunologic Pathogenesis

This disorder became a prominent dermatologic problem following the introduction of germicidal soaps in the late 1950s and early 1960s. Several chemicals, particularly halogenated salicylanilides and bithionol, were found to be prime offenders in producing sensitization. However, other drugs and chemicals such as chlorpromazine and sulfonamides have also been found to produce photoallergic contact dermatitis when applied topically either intentionally or accidentally. Recent evidence indicates that a component of musk deodorant is capable of inducing a photoallergic contact dermatitis.

The role of ultraviolet light in the production of photoallergic contact dermatitis is controversial. Evidence has been presented that ultraviolet light causes photodecomposition of topically applied chemicals and in the process generates a potent sensitizing contact antigen which, when applied to the skin of a sensitized individual in the absence of ultraviolet light, produces an eczematoid reaction. An alternative hypothesis is that ultraviolet light forms free radicals which generate a strong covalent bond between the chemical contactant and host skin structures and that binding of the chemical to the skin results in formation of the contact antigen.

Although the exact role of ultraviolet light is still

unclear, there is little doubt about the immunologic nature of this eczematous disease. Photoallergic contact dermatitis has been passively transferred by the intraperitoneal injection of mononuclear cells from a sensitized guinea pig into an unsensitized one.

Clinical Features

Clinically, the diagnosis is made by observation of the characteristic eczematous involvement in areas of skin exposed to light and by a history of exposure to photocontactants. A special form of patch testing is employed to confirm the diagnosis. This procedure consists of applying the suspected agent to the patient's skin and then later—usually within 24 hours—exposing the patch test site to ultraviolet light. A second covered patch test serves as a control. An eczematous eruption at 48 hours at the patch test site represents a positive response. The reaction duplicates the clinical lesion.

Treatment

Topical and systemic corticosteroids, avoidance of the offending agent, and avoidance of sunlight have all been used with some success, but persistence or recurrence of the eczema (persistent light reactor syndrome, actinic reticuloid syndrome) may occur. There is evidence that persistence of the disease is due to prolonged retention of the offending agent in the skin, but this is controversial. Thus, despite lack of recent exposure to the antigen, patients can develop eczematous disease upon exposure to ultraviolet light.

DERMATOPHYTOSIS

Dermatophytes are saprophytic fungi capable of evoking an eczematous response on infecting the skin. They commonly produce infection in the groin (jock itch), on the fingernails and toenails, and between the toes (athlete's foot). Studies by Jones et al indicate that an intact cell-mediated immune response may play an important role in the normal host's response to dermatophyte infections and may be responsible for the clinical manifestations of the skin lesions. These authors have demonstrated that the experimental inoculation of dermatophytes in the skin of an unsensitized individual results in propagation of the fungal disease (an eczematous lesion). However, within 48 hours the cutaneous fungal infection resolves. This resolution corresponds to the development of delayed hypersensitivity to the fungus. Subsequent infection of the arm of a sensitized individual results in a more intense transient initial eczematous response with persistent failure of the fungal infection to spread.

Fungal infections of the feet can be readily treated by the use of various topical preparations, including undecylenic acid powder, 2% miconazole cream, and tolnaftate solution or cream. Severe cases require the use of microsize griseofulvin, 0.5–1 g orally daily for approximately 3 weeks.

Fungal infections are usually self-limited, involving only the moist areas of the body. However, generalized cutaneous fungal infections, including tinea versicolor, have been reported in immunosuppressed patients, in patients with Hodgkin's disease, and in patients with isolated defects in cell-mediated immunity.

MUCOCUTANEOUS CANDIDIASIS

Major Immunologic Features

- Syndrome of cutaneous candidiasis generally found in the presence of T cell dysfunction.
- T cell defects include lymphocytopenia, the presence of lymphocytotoxin, serum factors inhibiting lymphocyte activation, absence of a 48-hour delayed skin test response to a variety of antigens, and impaired lymphocyte transformation or MIF production.
- Neutrophil chemotactic defects may be present.

General Considerations

Mucocutaneous candidiasis is a rare syndrome associated with skin and mucous membrane infection with *Candida albicans* that occurs most commonly—though not exclusively—in patients with defective cell-mediated immunity. It has been found in patients suffering from Hodgkin's disease and thymomas, in children born with severe combined immune deficiency, and in the DiGeorge and Nezelof syndromes. This form of candidal infection has also been found in children born with ill-defined and subtle profound defects in function of the T lymphocyte system.

C albicans, a saprophytic yeast, is a common inhabitant of the human gastrointestinal tract. Exposure begins in early infancy. During pregnancy, approximately 25% of women have significant yeast vaginitis. Approximately 5% of newborn infants develop oral candidiasis (thrush of mouth). Oral candidiasis generally disappears during the neonatal period. By the end of the first year of life, almost all children have been exposed to *C albicans*, as indicated by the presence of serum *Candida*-agglutinating antibodies. One study also indicates that approximately 60% of children over 1 year of age can mount a 48-hour delayed response to the intradermal injection of *C albicans* skin test antigen.

Children with mucocutaneous candidiasis may also have other fungal infections. However, *C albicans*, by virtue of its colonization of the gut in infancy, is the first potentially pathogenic fungus to challenge children with defective cell-mediated immunity.

Immunologic Pathogenesis (See Chapter 20.)

Extensive immunologic studies of patients with mucocutaneous candidiasis have demonstrated a variety of abnormalities of the cell-mediated immune mechanism. Most patients are unable to respond with a 48-hour delayed skin reaction to a battery of skin tests. This cutaneous "anergy" appears on infrequent occasions to be limited to the *Candida* antigen. These

patients also have delayed homograft rejection and an inability to become sensitized with DNCB. Some in vitro studies have demonstrated lymphocyte failure to respond to an antigenic challenge with increased DNA synthesis and migration inhibitory factor (MIF) production. Other patients' lymphocytes appear to have normal MIF production, but lymphocyte proliferation does not occur in response to antigenic challenges. On the other hand, some patients have a normal lymphocyte proliferative response but fail to produce additional MIF.

Mucocutaneous candidiasis has also been reported in association with a serum inhibitory factor that prevents lymphocytes from proliferating in response to antigenic challenge in vitro. One report describes clearance of the inhibitory factor and return of cell-mediated immunity following successful eradication of the *C albicans* infection with amphotericin B. This suggests that this serum inhibitory factor may be secondary to the *Candida* infection.

Recent studies also suggest that some of these patients may have defective neutrophil and mononuclear chemotaxis in addition to a defective cell-mediated immunity.

Clinical Features

Mucocutaneous candidiasis generally develops during the first 2 years of life. It commonly presents as persistent oral candidiasis beyond the neonatal period. The candidiasis may spread to involve large areas of skin. Granulomatous lesions may or may not form. The esophagus and nails may be involved. Multiple endocrinopathies and, at times, iron deficiency anemia may also be present.

Immunologic Diagnosis

The diagnosis of mucocutaneous candidiasis is made by culturing *C albicans* from the cutaneous lesions. For information regarding in vitro lymphocyte testing to evaluate the suspected underlying cell-mediated defect, see Chapter 18.

Differential Diagnosis

The granulomatous lesions of mucocutaneous candidiasis must be differentiated from pyodermas, cutaneous coccidioidomycosis, histoplasmosis, blastomycosis, and leishmaniasis.

Treatment

Some children with mucocutaneous candidiasis have been successfully treated by immunologic reconstitution with transfusions of normal homologous leukocytes. Thymic transplantation and amphotericin B, followed by transfer factor therapy, have also been successfully employed. Topically, 2% miconazole cream and clotrimazole have both been successfully employed.

Recent data indicate that some patients with mucocutaneous candidiasis may be treated with the histamine (H_2) antagonist cimetidine. Although the data are preliminary, they suggest that histamine and other inflammatory mediators may—in addition to their phlogistic activity—modulate the immune system. For example, suppressor T cells stimulated by concanavalin A possess H_2 receptors. Further research with cimetidine certainly appears warranted and offers an exciting new approach to the treatment of some defective T cell-mediated responses.

Prognosis

The prognosis is guarded. Theoretically, defective T cell function makes these individuals susceptible to viral, fungal, and protozoal infections as well as autoimmune disorders.

BULLOUS DISEASES

The bullous diseases can only be properly understood if the distinctive anatomic features of the skin are clearly visualized. The epidermis is composed of layers of epidermal cells. These cells originate in the basal layer of the epidermis and then migrate toward the stratum corneum. During this migration, the cells undergo a process of keratinization. Upon reaching the skin surface, these compacted keratinized epidermal cells form the **stratum corneum**, or **horny layer**, of the skin.

Individual epidermal cells, during their upward migration, are held together by desmosomes ("intercellular bridges"). These epidermal cells also interdigitate with one another, sharing narrow intercellular spaces filled with the so-called intercellular "cement." Destruction of the intercellular cement interferes with the cohesion of the epidermis, leading to blister formation.

The epidermis is anchored to the dermis by undefined forces. However, the basal cell layer adheres to the dermal **lamina densa (basal lamina)** by means of proteins confined to the **lamina lucida** (the space separating the basal cell membrane from the lamina densa) and **hemidesmosomes.** Destruction of the integrity of the basement membrane and surrounding structures results in separation of epidermis from the dermis and blister formation (Table 29–1).

SCALDED SKIN SYNDROME

The scalded skin syndrome (Ritter's disease, Lyell's disease) is a relatively benign acute febrile disease of infancy and early childhood characterized by mucopurulent rhinorrhea, conjunctivitis, mild to moderate toxemia, and a cutaneous response characterized by fragile intraepidermal blister formation and the loss of sheets of epidermis following minimal shearing pressure (Nikolsky's sign). Group II phage 71 *Staphylococcus aureus* can be isolated from the nose or conjunctiva. The cutaneous disease is short-lived, and recovery is complete.

Histologic and electron microscopic studies reveal epidermal cleft formation in the subgranular layer re-

Table 29–1. Immunologic studies in bullous skin diseases.

Disease	Site of Blister Formation	Immunoelectron Microscopic Localization of Immunoglobulin and Complement Deposits	Pattern and Immunoglobulin Isotypes	Serum Autoantibodies
Pemphigus	Intraepidermal	Squamous intercellular space	Linear IgG	Yes (90%)
Bullous pemphigoid	Subepidermal	Lamina lucida	Linear IgG	Yes (80%)
Benign mucous membrane pemphigoid	Subepidermal	Lamina lucida	Linear IgG	Yes (10%)
Herpes gestationis	Subepidermal	Lamina lucida	Linear IgG (25%)	Yes (25%)
Dermatitis herpetiformis	Subepidermal	Sub lamina densa	Granular IgA (~100%)	No
Linear IgA dermatosis	Subepidermal	Lamina lucida (predominantly), a few sub-lamina densa	Linear IgA	Yes (occasionally)
Bullous disease of childhood	Subepidermal	Lamina lucida	Linear IgA	Yes (occasionally)
Epidermolysis bullosa acquisita	Subepidermal	Sub-lamina densa, anchoring fibril zone	Linear IgG	Yes (occasionally)
Erythema multiforme	Subepidermal	Dermal blood vessels	Granular IgM	No
Staphylococcal scalded skin syndrome	High intraepidermal	None	None	No
Toxic epidermal necrolysis	Low intraepidermal	None	None	No

sulting from intraepidermal separation. Epidermal cytotoxic changes are minimal.

Experiments in newborn mice employing a low-molecular-weight protein (MW approximately 25,000), exfoliatin, isolated from group II phage 71 *S aureus* filtrates, have demonstrated that the intradermal injection of this protein produces epidermal denudation similar to if not identical with that seen in human infants and small children. Further studies in humans have demonstrated the presence of a detectable serum antibody to the exfoliatin found in the recovery phase of the disease. This antibody then appears to confer host immunity against subsequent toxic epidermal necrolysis cutaneous response to staphylococcal group II infections.

On rare occasions the exfoliatin (epidermolysin) toxin may be found in non-group II staphylococcal infections.[1]

PEMPHIGUS VULGARIS

Major Immunologic Features

- Immunoglobulin and complement deposition found in the squamous intercellular spaces.
- Serum antibody directed against intercellular substance of stratified squamous epithelium.
- Increased incidence of HLA-DRw4.
- Pemphigus IgG promotes epidermal cell detachment in vitro.
- Pemphigus IgG passively transferred to mice induces skin disease.

General Considerations

Pemphigus vulgaris is a chronic bullous disease which formerly was reported almost exclusively in Jews. It has now been documented in all races and ethnic groups. Pemphigus vulgaris has been reported in association with bullous pemphigoid, thymoma, myasthenia gravis, and systemic lupus erythematosus. Before the corticosteroid and antibiotic drugs became available, pemphigus vulgaris was fatal in a large percentage of cases. Patients died of fluid and electrolyte abnormalities, cachexia, and sepsis secondary to the denudation of large areas of skin.

New studies indicate that 91% of Jewish patients with pemphigus possess the HLA-DRw4 phenotype, compared to a 25% prevalence in controls. Furthermore, the A26, Bw38, DRw4 haplotype is almost nonexistent in non-Jewish whites but has a frequency of 11% in the normal Jewish population. However, this haplotype has a 36% prevalence in Jewish pemphigus patients. The Bw38 and DRw4 phenotypes occur jointly in 55% of Jewish pemphigus patients and in 11% of controls.

It is also interesting to note that the DRw4 phenotype was found in 4 of 4 Mexican, 3 of 3 Oriental, and 7 of 17 non-Jewish white pemphigus patients. The meaning of this striking increased prevalence of the DRw4 phenotype in pemphigus patients is unknown.

Immunologic Pathogenesis

Direct immunofluorescence of the skin lesions in pemphigus vulgaris has demonstrated the deposition of immunoglobulins (predominantly IgG), complement components (C1, C4, C3), properdin factor B (C3 proactivator), and, to a lesser extent, properdin at the site of involvement, the epidermal intercellular spaces. In addition to evidence of complement deposition at the disease site, complement levels in the blister fluid—in contrast to serum complement levels—are markedly decreased. Preliminary evidence also indicates the presence of C1q precipitating material in the blister fluid, suggesting the presence of immune complexes. All of the above studies have been interpreted as suggesting local activation of complement at the site of blister formation.

However, in vitro studies using normal human skin

explants show that pemphigus antibody in the apparent absence of complement can also induce acantholytic epidermal lesions. Additional in vitro studies have shown that pemphigus IgG promotes epidermal cell detachment in primary epidermal cell cultures. This detachment process may be the result of activation of certain proteases, ie, plasminogen activator or other unknown enzymes.

Conclusive evidence exists that the pemphigus antibody is responsible for the induction of pemphigus. Anhalt et al have passively transferred pemphigus to neonatal mice by intraperitoneal injections of IgG from pemphigus. These animals develop a cutaneous blistering disease clinically, histologically, and immunologically similar to human pemphigus. At present, the evidence strongly supports the hypothesis that pemphigus is an autoimmune disease in which the pemphigus autoantibodies are responsible for inducing epidermal acantholysis and bulla formation. Following the binding of pemphigus antibody to the keratinocyte cell surface antigen (receptor?), these cells detach from each other. In vitro studies suggest that this detachment process is associated with the release of proteases that in turn can promote cell detachment.

A pemphigus foliaceus-like skin disease occurs in 5–10% of rheumatoid arthritis patients receiving penicillamine for 6 or more months. Furthermore, these patients demonstrate classic pemphigus antibodies in perilesional biopsies and in their serum. Discontinuation of penicillamine is generally followed by disappearance of clinical and serologic findings of pemphigus, although some patients require treatment. It is also interesting to note that pemphiguslike antibodies have been described in skin lesions associated with sensitivity to penicillin and ampicillin.

Most (not all) specimens of serum from pemphigus patients contain a circulating IgG antibody (Fig 29–1) directed against a substance in the intercellular spaces of squamous epithelium.

Clinical Features

Pemphigus vulgaris is characterized by the development of thin flaccid bullae on normal-appearing skin. The mucous membranes are commonly involved. In fact, the disease commonly begins with extensive oral erosions. Shearing force applied to normal-appearing skin or direct pressure applied to the blister will cause further denudation of the skin and extension of the blister (Nikolsky's sign). The ruptured blisters display little tendency to heal.

Histologic examination of the blister reveals intraepidermal blister formation (the epidermis forms both the roof and base of blisters). In the blister fluid one can see single, unattached, rounded epidermal cells (Tzanck cells). At the margin of the blister, intercellular clefts may be seen between the individual epidermal cells (acantholysis).

Electron microscopic studies have demonstrated that destruction of the intercellular "cement" substance is the earliest pathologic finding in pemphigus vulgaris.

Figure 29–1. Indirect immunofluorescence examination of pemphigus serum, demonstrating the presence of IgG antibody to intercellular substance. The substrate is monkey esophagus.

Immunologic Diagnosis

The diagnosis is made by the clinical features and histologic demonstration of acantholytic intraepidermal bulla formation. The diagnosis of pemphigus can also be made by the direct immunofluorescence demonstration of immunoglobulin deposition in the intercellular spaces of diseased or normal-appearing epidermis of the patients. This can also be confirmed by demonstrating the typical pemphigus antibody in the patient's serum by indirect immunofluorescence techniques that employ heterologous tissue as a substrate.

Differential Diagnosis

This disease must be differentiated from other blistering diseases such as bullous pemphigoid, erythema multiforme, and benign mucous membrane pemphigoid.

Treatment

This disease can be successfully controlled with high doses of corticosteroids. In the past, prednisone in doses as large as 300–500 mg/d was required to control the lesions. In recent years, either azathioprine, 100–150 mg orally daily, methotrexate, 25–50 mg intramuscularly once weekly, or cyclophosphamide, 50–100 mg orally daily, usually in conjunc-

tion with corticosteroids, 40–60 mg orally daily, has been employed to treat these patients. The combined use of corticosteroids with immunosuppressive therapy allows one to employ much smaller doses of the former, thus avoiding the objectionable complications of corticosteroid therapy.

Recent evidence also suggests that intramuscular gold therapy is effective in the treatment of pemphigus. This form of therapy appears to be especially helpful in the treatment of patients with complications from corticosteroids or a poor response to corticosteroids.

After prolonged therapy, about 30–40% of pemphigus patients undergo a sustained clinical and serologic remission that persists without further therapy. Complications of corticosteroid and immunosuppressive therapy are the major clinical problems in the treatment of pemphigus, accounting for 8–10% of deaths in this disease.

Prognosis

The prognosis of this disease has changed radically in the last 2 decades. Death during the first or second year after onset occurred in approximately 50% of patients in the preantibiotic and precorticosteroid era. The mortality rate is now being reported as 8% in several studies. However, morbidity (gastrointestinal bleeding, osteoporosis, diabetes) associated with large doses of corticosteroids is high.

BULLOUS PEMPHIGOID

Major Immunologic Features

- Bullous disease common in elderly patients.
- Separation of epidermis from dermis at the level of the lamina lucida.
- Immunoglobulin and complement deposition on the skin basement membrane.
- Serum anti-skin basement membrane antibody found in approximately 80% of patients.
- Passive in vivo transfer of the disease with human pemphigoid antibody.
- Neutrophil and eosinophil chemotactic factors in blister fluid.

General Considerations

This is a chronic bullous disease occurring mostly in middle-aged and older people. Like pemphigus vulgaris, this blistering disease had a significant mortality rate prior to the advent of corticosteroids and antibiotics.

Clinical Features

Bullous pemphigoid is a self-limited blistering disease characterized by formation of tense bullae on an erythematous base. The flexor areas of the body (axillary, inguinal, and sides of the neck) are the common sites of involvement. The blister forms subepidermally. (The roof is formed by the epidermis, and the base of the blister is the dermis.) The individual bullae are difficult to rupture. Mucous membrane lesions are not a common feature. It has recently been recognized that bullous pemphigoid may occur as isolated bullae localized to one area of the body, especially the lower legs. Patients with isolated bullous lesions demonstrate classic histologic and serologic bullous pemphigoid features.

Immunologic Pathogenesis

Direct immunofluorescence studies have demonstrated the deposition of IgG, IgA, IgM, IgD, IgE, C1q, C4, C3, C5, properdin, properdin factor B, β_1H, and fibrin along the skin basement membrane (the site of bullous formation). Direct immunoelectronmicroscopic studies demonstrate localization of IgG and C3 in the lamina lucida.

There is no increased incidence of a particular HLA phenotype, nor is there an increased incidence of malignancy associated with bullous pemphigoid.

Complement component levels in the blister fluid are markedly decreased, whereas the total serum complement in these patients is normal. As in pemphigus vulgaris, these studies have been interpreted as suggesting local activation of complement at the site of blister formation.

Eosinophil and neutrophil chemotactic activity has been demonstrated in the blister fluids. One of the neutrophil chemotactic factors is C5, and at least one of the eosinophil chemotactic factors closely resembles eosinophil chemotactic factor of anaphylaxis (ECF-A).

Recent studies suggest the presence of various proteolytic enzymes in the blister fluids of bullous pemphigoid patients. These enzymes have tosyl-arginyl-methylester (TAMe) esterase, Hageman factor cleaver, and elastase activity. Some of these enzymes may originate in the mast cell or neutrophil.

Morphologic studies also support a possible pathologic role for mast cells in the pathogenesis of bullous pemphigoid. One of the earliest events in the development of a bullous pemphigoid blister is the migration and lining up of mast cells along the basement membrane zone. Electron microscopic studies suggest that these mast cells are activated.

In addition to these studies, indirect immunofluorescence techniques (Fig 29–1) have demonstrated, in the sera of approximately 80% of these patients, a complement-fixing IgG antibody to skin basement membrane. The antigens that are recognized by pemphigoid autoantibodies are large-molecular-weight proteins localized on the attachment plaque of basal cell hemidesmosomes of squamous epithelia. The role of pemphigoid autoantibodies in the biology of the hemidesmosome is under active study at the present time.

Studies by Gammon employing a unique in vitro tissue culture system have demonstrated that human skin cultured in the presence of pemphigoid antibody, complement, and human leukocytes displays a directed migration and attachment of leukocytes to the skin basement membrane zone. This directed migra-

tion is an active process requiring viable leukocytes and complement. Following attachment of these leukocytes to the skin basement membrane zone, there is subepidermal separation. This constitutes the first in vitro evidence that the pemphigoid antibody is indeed responsible for the development of the subepidermal blistering disease so characteristic of bullous pemphigoid.

Anhalt and Diaz have developed a rabbit model for bullous pemphigoid using corneal epithelium (which shares pemphigoid antigen with the epidermis) as target for purified IgG from bullous pemphigoid. IgG from pemphigoid injected in the stroma of rabbit cornea induced a severe keratitis with subepithelial vesiculation and binding of pemphigoid antibodies to the epithelial-stromal junction. The inflammatory response triggered in the cornea by IgG from pemphigoid correlated with the doses of IgG injected and the complement fixation properties of the pemphigoid antibodies.

Immunologic Diagnosis

The diagnosis is made clinically by observing the typical clinical features and by demonstrating a subepidermal bulla on a histologic preparation. The diagnosis may also be established by demonstrating, by direct immunofluorescence, the deposition of immunoglobulins or complement (C3) along the skin basement membrane (Fig 29–2). The serum may also be employed in indirect fluorescence techniques to demonstrate IgG skin basement membrane antibodies.

Figure 29–2. Direct immunofluorescence examination of the skin of a patient with bullous pemphigoid. Note heavy immunoglobulin deposition along the basement membrane.

Differential Diagnosis

This bullous disease must be differentiated from pemphigus vulgaris and erythema multiforme. The small blisters of an acute onset of bullous pemphigoid may be confused with dermatitis herpetiformis.

Treatment

Like pemphigus vulgaris, bullous pemphigoid responds to high doses of corticosteroids (eg, prednisone, 40–60 mg orally daily). Azathioprine, 100–150 mg orally daily, and methotrexate, 25–50 mg intramuscularly once weekly, have also been successfully employed, along with corticosteroids, in the treatment of bullous pemphigoid. As in pemphigus vulgaris, the combined use of corticosteroids and immunosuppressive agents has allowed use of low doses of corticosteroids, thereby reducing the incidence of severe corticosteroid complications.

Prognosis

The prognosis for life is much improved, although, as in pemphigus vulgaris, large doses of corticosteroids in elderly people involve great risks that can only be undertaken if the probable benefits outweigh the possible hazards.

The great majority of patients with bullous pemphigoid have a self-limited disease. The average course of immunosuppressive or steroid therapy is 4–6 months. Thereafter, most patients have a prolonged (over 2 years) clinical, immunohistologic, and serologic remission.

BENIGN MUCOUS MEMBRANE PEMPHIGOID

This is a subepidermal blistering disease involving the mucous membranes of the eyes, mouth, and vagina. Scar formation may occur. Few or no blisters appear on the skin. Recent investigations have now firmly established the presence of immunoglobulins and complement components along the basement membrane of the mucous membrane (the site of blister formation). One recent study demonstrated an 80% incidence of immunoglobulin or complement deposition along the basement membrane zone of perilesional biopsies in 25 patients. Serum anti-basement membrane zone antibodies were detected in approximately 10% of patients. These observations suggest a possible relationship between this disease and bullous pemphigoid.

Treatment

Treatment of this disease is difficult. Local forms of therapy with corticosteroid eye drops and surgical procedures do not produce lasting benefits. There is

some evidence that these lesions respond partially to systemic corticosteroid therapy (eg, prednisone, 40–60 mg orally daily). The benefits of corticosteroid therapy must be weighed against the possible serious side effects of corticosteroids in elderly persons. Immunosuppressive therapy (azathioprine, 100–150 mg orally) has been used in some of these patients, although its efficacy remains to be proved.

Prognosis

Benign mucous membrane pemphigoid is a chronic scarring disease of mucous membranes. Conjunctival scarring leads to failure to close the eye. Severe dryness and infection follow, and ultimately blindness.

HERPES GESTATIONIS

Major Immunologic Features

- Bullous dermatosis of pregnancy.
- May be exacerbated or precipitated by estrogen-containing birth control pills.
- Complement and, at times, immunoglobulin deposition on skin basement membrane.
- Avid complement-fixing serum autoantibody.

General Considerations

Herpes gestationis is a rare subepidermal bullous disease of pregnancy, occurring in one out of every 10–30 thousand pregnancies. The term herpes in this disease does not refer to herpesviruses but to the serpentine appearance of the lesions. It is characterized by an intense burning pruritus and subepidermal blister formation on erythematous papules. Histologically, the blister formation is indistinguishable from that of bullous pemphigoid.

Herpes gestationis may occur at any time during pregnancy but occurs most commonly in the late second or early third trimester. It disappears following the termination of pregnancy, but persistence into the postpartum period and episodic flares with menses have been reported. The disease has also been reported following the use of estrogen-containing birth control pills. It may recur in succeeding pregnancies and may have an earlier and more severe onset. Infants born of affected mothers are generally without evidence of skin disease, but on rare occasions a transient bullous skin disease has been reported.

Immunologic Pathogenesis

Direct immunofluorescence studies of the skin of patients with herpes gestationis have demonstrated IgG, IgE, C1, C4, C3, C5, and properdin deposition along the basement membrane. In many cases biopsy shows only heavy C3 deposition. The serum may or may not contain a factor capable of depositing complement components C1, C4, or C3 on the skin basement membrane. While some technical difficulty has been encountered in demonstrating a serum anti-basement membrane autoantibody in some patients, it appears likely that this basement membrane complement-fixing serum factor is an immunoglobulin.

Immunologic Diagnosis

Direct immunofluorescence studies of the skin of infants afflicted with the disease have thus far revealed the deposition of only C4 and C3 on the basement membrane. Immunoelectronmicroscopic studies indicate that immunoreactants are localized to the lamina lucida in a pattern quite similar to that found in bullous pemphigoid.

These studies suggest a role for immunoglobulin and complement in the pathogenesis of the bullous disease of the mother and infant. The relationship between this disease and bullous pemphigoid is unknown, although immunofluorescence staining of specimens of basement membrane gives quite similar results.

Treatment

Treatment of herpes gestationis is difficult. These patients complain of an intense pruritus in addition to the blistering skin disease. The disease can be controlled during pregnancy with low to moderate doses of prednisone (15–30 mg orally daily). The aim is to control the disease and make the patient comfortable until she delivers. One possible complication is that the prednisone may suppress the fetus' adrenals. The neonatologist should be warned of the possibility of adrenal insufficiency in the infant at birth.

Prognosis

Generally, the bullous disease disappears within a few months after termination of pregnancy. Exacerbations may occur with subsequent menses or with the use of birth control pills. The mechanism of induction by birth control pills is completely unknown. Subsequent pregnancies may result in a more acute and earlier onset of the bullous disease. Recent evidence suggests that there may be an increased incidence of neonatal deaths among infants born to mothers with herpes gestationis.

DERMATITIS HERPETIFORMIS

Major Immunologic Features

- Granular deposition of IgA at the dermal-epidermal junction.
- Increased incidence of HLA-B8 antigen.
- Gluten-sensitive enteropathy.
- Gluten-free diet or sulfones induce remissions.
- IgA immune complexes in serum.

General Considerations

Dermatitis herpetiformis is a chronic bullous disease that may occur at any age. Unlike pemphigus and, to a lesser extent, bullous pemphigoid, untreated dermatitis herpetiformis is not fatal.

Immunologic Pathogenesis

Direct immunofluorescence studies show that granular deposition of IgA is almost always present at the

dermal-epidermal junction and IgG and IgM deposition much less commonly so. C3 is often found at sites corresponding to IgA deposition, especially in areas of blister formation. C1q and C4 are only occasionally found. These studies have been interpreted as suggesting activation of complement, predominantly via the alternative pathway, at the site of blister formation. No serum autoantibodies have been demonstrated, and serum complement levels are normal. Immunoelectronmicroscopy demonstrates the sub-lamina lucida granular deposition of immunoreactants.

Genetic studies have demonstrated that approximately 90% of patients with dermatitis herpetiformis have HLA-B8 and HLA-Dw3 antigen, compared to a frequency of less than 30% in the general population. About 90% of patients suffering from adult celiac disease also have HLA-B8. Interestingly, the majority of patients with dermatitis herpetiformis demonstrate gluten-sensitive enteropathy. However, patients with adult celiac disease do not show IgA deposition in their skin.

The relationship between the gut disease and the skin disease is unknown. This may be a hypersensitivity disease in genetically predisposed individuals. Conceivably, antigen—perhaps gluten—is presented to the host via the gastrointestinal tract, preferentially stimulating the secretory immune system (IgA). Combining the antigen with IgA in the gut could produce the patchy duodenal or jejunal atrophy. The damaged mucosa may then permit IgA immune complexes to diffuse into the systemic circulation. For unknown reasons, these IgA complexes may be deposited in the skin, where they activate the complement system via the alternative pathway and produce the skin disease. Another hypothesis is that the inciting antigen and a normal skin structure—perhaps reticulin fibers—may bear antigenic similarities to each other. The IgA directed against the inciting antigen may then cross-react with the skin structure.

Recent evidence indicates the cutaneous manifestations as well as the gut lesions in dermatitis herpetiformis respond to a gluten-free diet. Another study has indicated that there is a concomitant disappearance of the skin lesions and IgA deposition following institution of a gluten-free diet.

Dermatitis herpetiformis patients have been shown to possess antigliadin antibodies. Three groups of investigators have demonstrated the presence of serum IgA immune complexes in approximately one-third of the random sera of dermatitis herpetiformis patients. Dermatitis herpetiformis patients contain little or no serum IgG immune complexes.

Clinical Features

Dermatitis herpetiformis is characterized by small groups of tense vesicles on an erythematous base. Symmetric distribution over the extensor surfaces is common, and the buttocks, lower back, and shoulders are most severely affected. An intense burning pruritus accompanies this disease.

Histologically, the lesions demonstrate a subepidermal bullous formation. Eosinophilic microabscesses are seen in the dermal papillae.

Dermatitis herpetiformis also has systemic manifestations. Biopsies of the small intestine have revealed a patchy duodenal or jejunal atrophy indistinguishable from adult celiac disease. Signs and symptoms of a malabsorption syndrome, however, are only occasionally seen (probably because of the patchy involvement of the gut disease).

Immunologic Diagnosis

In addition to the characteristic clinical and histologic features of this disease, the direct immunofluorescence demonstration of granular IgA deposition in the upper dermis of these patients is considered diagnostic of dermatitis herpetiformis. IgA deposits are present in over 95% of patients upon random biopsy of normal skin. Recent evidence indicates that approximately 10% of dermatitis herpetiformis patients have linear rather than granular IgA deposition along the dermal-epidermal junction. The majority of these patients, upon immunoelectronmicroscopic examination, demonstrate sub-lamina lucida deposition of the immunoreactants.

Differential Diagnosis

Dermatitis herpetiformis must be differentiated from other intensely pruritic diseases such as scabies, pediculosis, and neurodermatitis and the blistering disease bullous pemphigoid.

Treatment

Dapsone (diaminodiphenylsulfone), 100 mg daily, and sulfapyridine, 1–3 g daily, are effective in controlling the cutaneous features of this disease. They have no effect on the gastrointestinal lesions. The mechanisms of action of these drugs are unknown. The gut lesions respond to a gluten-free diet, and in one recent report it was claimed that a prolonged gluten-free diet resulted in clearance of the skin lesions.

Prognosis

The prognosis is excellent. Unlike in bullous pemphigoid and pemphigus vulgaris, no deaths occur as a result of this disease. Untreated, it persists for years and is characterized by chronic low-grade activity with acute exacerbations. Patients treated with dapsone and free of disease for years, upon discontinuation of the dapsone, may have recurrence of their disease within 72–96 hours.

IgA BULLOUS DERMATOSIS

Bullous dermatosis is a newly recognized entity characterized by subepidermal bullous formation on glabrous skin. Direct immunofluorescent studies demonstrate a linear deposition of IgA along the zone of the basement membrane. Immunoelectronmicroscopy shows deposition of IgA in the lamina lucida

in a pattern similar to that of bullous pemphigoid. Several of these patients have IgA anti-basement membrane antibody in their serum. These patients—like dermatitis herpetiformis patients—respond to sulfones; unlike dermatitis herpetiformis patients, however, they do not have an increased incidence of HLA-B8 or HLA-Dw3 phenotypes or a patchy duodenojejunal atrophy.

Patients with this peculiar bullous disease have undoubtedly been diagnosed as having linear IgA dermatoses and linear IgA dermatitis herpetiformis.

CHRONIC BULLOUS DISEASE OF CHILDHOOD

The chronic, subepidermal bullous disease of children is characterized by prominent flexural bullous lesions, especially in the inguinal region. In most (but not all) cases, direct immunofluorescence is negative. In other cases, there is linear deposition of IgA along the basement membrane zone.

Many of these children respond to sulfones or sulfapyridine. The relationship of this childhood entity to adult IgA bullous disease is unknown.

EPIDERMOLYSIS BULLOSA ACQUISITA

This is an uncommon subepidermal vesicular bullous disease of the skin and the mucous membranes. Traumatic induction of blister formation and mucous membrane blisters are prominent findings. Recent direct immunofluorescent studies have demonstrated bound immunoglobulin and complement deposited in a linear configuration along the basement membrane zone. In addition, several of these patients have an IgG antibody in their serum which reacts with the basement membrane zone. Electron microscopic studies, however, have demonstrated that the blister formation occurs below the lamina densa. Yaoita et al, employing immunoelectronmicroscopic techniques, have demonstrated that the bound IgG and the circulating IgG antibodies found in some of these patients are reactive against antigens localized to the sub-basal lamina zone. The antigen recognized by the autoantibodies in these patients is a large-molecular-weight dermal, insoluble protein. The exact relationship of this protein with collagen remains to be determined.

Most importantly, these studies demonstrate that despite a similarity of this blistering disease with bullous pemphigoid, the 2 diseases represent distinct clinical and pathologic entities. Furthermore, in marked contrast to bullous pemphigoid, this blistering disease is resistant to corticosteroid and immunosuppressive therapy.

Studies by Briggaman and Gammon have revealed that approximately 10% of patients clinically diagnosed as having bullous pemphigoid possess serum autoantibodies similar to those seen in epidermolysis bullosa acquisita. These studies suggest that epidermolysis bullosa acquisita may not be so rare as once thought.

ERYTHEMA MULTIFORME

Erythema multiforme is a common cutaneous reaction pattern seen in a variety of disorders, including infections, drug reactions, connective tissue diseases, and malignant or chronic disease of the internal organs. Recent data indicate that biopsies taken from early lesions of erythema multiforme frequently show deposition of granular deposits of C3 or IgM (or both) in blood vessels of the papillary dermis. IgA and IgG are detected only rarely in these specimens. About half of erythema multiforme patients with immunoglobulin and complement deposition in the papillary blood vessels had recurrent herpes simplex (herpesvirus hominis); in the remainder, a definite cause of erythema multiforme could not be found. In addition to these interesting findings, other studies in patients with the bullous form of erythema multiforme have recently demonstrated immune complexes and reduced levels of individual complement components in the blister fluid. Serum immune complexes have also been detected.

These data suggest a role for immunoglobulin and complement, perhaps in the form of immune complexes, in the pathogenesis of erythema multiforme. These data also provide further evidence that cutaneous manifestations interpreted as erythema multiforme are occasionally another cutaneous manifestation of an immune complex-mediated vascular insult.

Toxic epidermal necrolysis is a potentially fatal bullous disease characterized by blister formation occurring low in the epidermis. Widespread denuding of the skin can occur. Toxic epidermal necrolysis is frequently seen as a complication of a drug reaction. This skin reaction pattern is now thought to be related to erythema multiforme. Treatment consists of giving large doses of corticosteroids (eg, 60 mg of prednisone orally daily).

VASCULITIDES

Major Immunologic Features

- Multisystem inflammatory disease of blood vessels (skin, joints, gut, kidney, etc).
- Inflammatory purpuric lesions in skin (papules, bullae, ulcers).
- Deposition of immunoglobulin and complement in blood vessel walls.
- Serum complement levels may be decreased.
- Cryoglobulins and rheumatoid factor may be present in serum.

General Considerations

A partial list of vasculitic diseases includes leukocytoclastic angiitis, allergic granulomatosis, poly-

arteritis nodosa, giant cell arteritis, and Wegener's granulomatosis.

All forms of vasculitis may have significant cutaneous features. The size of the vessel, its anatomic location, and the intensity of the inflammatory insult determine what the cutaneous manifestations will be. Vasculitis may therefore present as pustular, petechial, urticarial, nodular, or ulcerative lesions. Recent studies have demonstrated deposits of various immunoglobulins, complement, and alternative pathway components in the diseased blood vessel walls of patients suffering from various forms of leukocytoclastic angiitis. Electron microscopic studies of blood vessel walls of early lesions of leukocytoclastic angiitis have demonstrated electron-dense deposits (presumably immune complexes). Immunoglobulin and complement are deposited in the temporal arteries of some patients suffering from temporal arteritis. Some patients with polyarteritis nodosa have deposits of HBsAg, immunoglobulins, and complement in the diseased blood vessel walls. In addition, circulating HBsAg antigen has been found in the serum of some of these patients.

These studies suggest that the deposition of immune complexes in the blood vessel walls may play a role in the pathogenesis of several forms of necrotizing vasculitis. Evidence also suggests that circulating immune complexes and complement activation may play a role in the pathogenesis of urticarial and angioneurotic edema-like lesions frequently seen in the prodromal stage of acute viral hepatitis.

Several studies have reported the association of recurrent urticaria, including angioedema, with marked hypocomplementemia involving both the classic and alternative complement pathways. Histologically, the urticarial plaques display a vasculitis. In contradistinction to hereditary angioneurotic edema, these patients have normal C1 esterase inhibitor activity and C1q is low. These patients have been described as having hypocomplementemic vasculitis. It is most important to stress that this is a systemic disease. Although a few patients have developed arthritis and renal disease, a large percentage—perhaps as high as 50%—have developed a rapid onset of progressive, potentially fatal obstructive lung disease. The nature of the lung abnormalities is not known. (See Chapter 21 for further clinical discussions of these disorders.)

A striking statistically significant association exists between Sjögren's syndrome and vasculitis. The vasculitis involves systemic organs, including the central nervous system, as well as the skin. The cutaneous manifestations of vasculitis found in association with Sjögren's syndrome range from urticarialike lesions to infarcts and ulcerations. These Sjögren syndrome patients with vasculitis have a statistically significant increased prevalence of rheumatoid factor, hypergammaglobulinemia, and Ro(SSA) and La(SSB) antibodies. A significant number of patients thought to have Waldenström's hypergammaglobulinemic purpura have been found to have occult Sjögren's syndrome and to possess Ro and La antibody.

The cutaneous vasculitic lesions and the glomerulonephritis which occur in patients with Henoch-Schönlein purpura are characterized by prominent deposits of IgA and terminal complement components. These patients also have been found to have IgA immune complexes in their sera.

Patients with the intestinal bypass surgery syndrome—an entity observed in patients who have undergone ileojejunal bypass surgery for obesity—characterized by deep dermal erythematous plaques and pustules and papules, possess serum IgA immune complexes. Work by Utsinger indicates that these complexes contain antigens related to enteric bacteria, eg, *Escherichia coli*.

Some patients with primary biliary cirrhosis have small cutaneous pustular lesions and possess in their serum large quantities of IgA immune complexes.

These studies suggest that some of the cutaneous manifestations associated with primary biliary cirrhosis, Henoch-Schönlein purpura, and intestinal bypass surgery syndrome may represent the cutaneous expression of an IgA-mediated immune complex vasculitis.

DISCOID LUPUS ERYTHEMATOSUS

Major Immunologic Features

- Lesions may or may not be associated with SLE.
- Immunoglobulins and complement components generally found in the dermal-epidermal junction in old discoid lesions or in uninvolved skin of patients with SLE (lupus band test).
- Photosensitivity.

General Considerations

The cutaneous lesions of discoid lupus erythematosus may be characterized as sharply demarcated atrophic plaques. Telangiectasia, follicular plugging, and a hyperkeratotic scale are often prominent. These lesions can involve any part of the body but are usually in light-exposed areas, especially the face and scalp. Histologically, the lesions are characterized by a patchy lymphocytic infiltrate at the dermal-epidermal junction. Liquefaction degeneration of the basal layer of the epidermis and epidermal atrophy are common features. These discoid lesions may be seen in the absence of any systemic disease or as part of the clinical picture of systemic lupus erythematosus (SLE).

Immunologic Pathogenesis

Direct immunofluorescence study of these lesions has revealed the deposition in a granular pattern of immunoglobulins, complement, and alternative pathway components at the dermal-epidermal junction. Elution studies have demonstrated that these immunoglobulins have antinuclear specificity. Antibodies to skin basement membrane have also been eluted from the granular deposits of the discoid skin lesions. However, the role of these granular deposits in the pathogenesis of discoid lesions is doubtful for 2 reasons: (1) Similar immunoglobulin and complement deposits are

found in normal-appearing light-exposed skin in approximately 60% of patients with SLE. These deposits have been found in patients who have never had any skin lesions. (2) Ultraviolet light is capable of inducing classic discoid lesions in patients with SLE. Direct immunofluorescence studies of these experimental ultraviolet light-induced lesions have failed to consistently demonstrate immunoglobulin or complement deposition. If deposition does occur, it is found only after the lesions are several months old.

Despite the lack of evidence that immune complexes play a role in the pathogenesis of the cutaneous lesions, their presence in the skin of patients with systemic lupus erythematosus that has not been exposed to light appears to be a reflection of the systemic immune complex disease. The presence of these cutaneous deposits is very often associated with severe lupus nephritis (Fig 29–3). Several studies have demonstrated a statistically significant correlation between noninvolved skin, immunoglobulin deposition at the dermal-epidermal junction (lupus band test), and the presence of serum hypocomplementemia, anti-DNA antibodies, and the presence of clinical renal disease. More recent evidence indicates that patients having either a negative lupus band test or a lupus band test composed of pure IgM have a lupus process characterized by a low incidence of renal disease. Patients possessing a lupus band test composed of IgG alone or together with other immunoglobulins, however, have a lupus process characterized by a high incidence of clinical renal disease and demonstrate serum hypocomplementemia and anti-DNA antibodies. Because of this association, the routine examination of the noninvolved and nonexposed skin of patients with SLE can provide valuable information regarding the presence or absence of immune complex nephritis.

It has recently been recognized that urticarialike lesions can occur in SLE as a manifestation of low-grade necrotizing vasculitis. Direct immunofluorescent examination of the urticarial lesions may reveal Ig and C3 deposition in the diseased blood vessels, and routine hematoxylin and eosin stains demonstrate a leukocytoclastic angiitis. Furthermore, systemic lupus erythematosus patients with these urticarial lesions appear to be acutely ill as well as chronically ill. They frequently manifest overt renal disease and demonstrate anti-DNA and anti-Sm antibodies, hypocomplementemia, and markedly elevated concentrations of immune complexes in their serum.

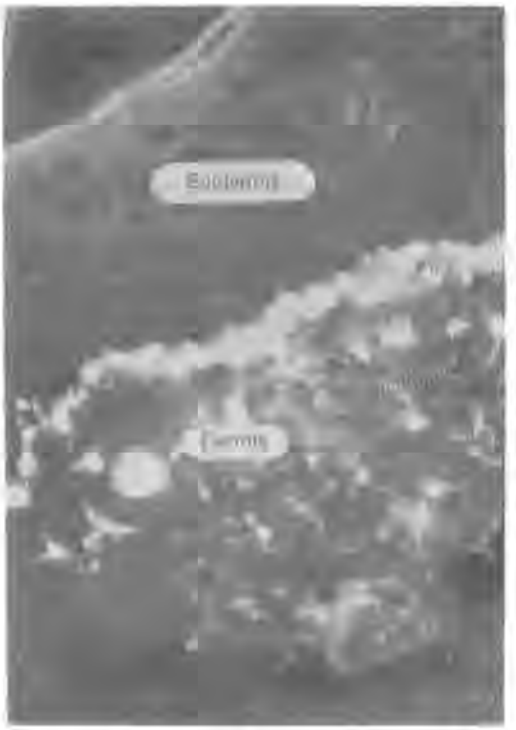

Figure 29–3. Direct immunofluorescence examination of the skin, not exposed to light, of a patient with systemic lupus erythematosus and severe nephritis. Note heavy granular deposition of IgM.

Immunologic Diagnosis

Several distinct lupus patient populations have now been described in whom prominent widespread, frequently annular polymorphic lupus lesions dominate the clinical picture and who demonstrate serologically the presence of **Ro(SSA)** antibodies. These patients frequently fail to demonstrate significant ANA titers or anti-native DNA (nDNA) antibodies. They have been variously diagnosed as having ANA-negative, subacute cutaneous, and neonatal lupus erythematosus. Similar patients with homozygous C2 and C4 deficiency, a lupuslike disease process, and **anti-Ro(SSA)** antibodies have also been identified.

The ANA-negative lupus erythematosus group of patients, in addition to having prominent photosensitive lupus dermatitis, frequently have systemic signs and symptoms, satisfying the American Rheumatism Association's criteria for the diagnosis of SLE. However, as is not the case with classic SLE, neurologic and renal diseases are unusual. About 70% of patients originally described as having ANA-negative SLE were Ro(SSA)-positive. Another 25%, although ANA- and Ro(SSA)-negative, possessed anti-single-stranded DNA (ssDNA) antibodies. Thus, these studies emphasize that although these patients failed to demonstrate significant ANA titers, they were not serologically negative. Our studies indicate that 5–10% of SLE patients are ANA-negative (mouse liver substrate). The presence of anti-Ro(SSA) and ssDNA antibodies provides a serologic relationship between these atypical, predominantly cutaneous SLE patients and classic SLE.

Antibodies against ssDNA have also been found in approximately 25% of patients with benign cutaneous lupus erythematosus. These antibodies are of the IgM

class. Most recent studies indicate that those benign cutaneous lupus patients with single-stranded DNA antibodies have an increased propensity to develop systemic disease.

A second group of cutaneous lupus patients with predominantly large annular, polycyclic, photosensitive lupus dermatitis have been described by Sontheimer et al as having subacute cutaneous lupus erythematosus. Approximately 50% of these patients satisfy the American Rheumatism Association's criteria for the diagnosis of SLE, and 70% demonstrate Ro(SSA) antibodies. These patients have a low incidence of renal disease. They demonstrate a statistically significant increased prevalence of the HLA-DR3 phenotype. Undoubtedly, they are part of the same spectrum of lupus patients described as having ANA-negative SLE.

Recent evidence also indicates that approximately 75% of homozygous C2- and C4-deficient patients with lupuslike disease are Ro(SSA)-positive. Clinically, these patients demonstrate large annular, polycyclic lupus lesions identical to those seen in subacute cutaneous lupus erythematosus. In addition, they frequently fail to demonstrate significant ANA titers and anti-nDNA antibodies. They generally demonstrate the A10-B18-DR2 haplotype.

In addition to patients with these atypical forms of lupus erythematosus with predominant skin disease who demonstrate Ro(SSA) antibodies, this antibody system has been detected in virtually all neonatal lupus infants and their mothers. Neonatal lupus is a rare syndrome characterized in its mildest form by the development of annular cutaneous lupus lesions. There is some evidence that these lupus lesions are induced by sun exposure. The lesions generally disappear by 6 months of age.

In its more severe forms, neonatal lupus may manifest various cardiac conduction defects in the absence of cutaneous lupus lesions. Scott et al and Lee et al have presented evidence that the mothers of the great majority of children born with isolated congenital heart block are Ro(SSA)-positive. Life-threatening complete heart block has necessitated cardiac pacemaker implantation in the neonatal period. Adams-Stokes syndrome has been reported in these patients during early infancy. In addition, preliminary studies indicate that these Ro(SSA)-positive mothers of infants with congenital heart block have an increased incidence of fetal wastage (stillborns and abortions). Thus, the spectrum of neonatal lupus extends from increased fetal wastage in utero to life-threatening cardiac conduction defects in infancy and early life.

The Ro(SSA) antibody disappears from the infant's circulation before 6 months of age but persists in the mother. These observations suggest that a serum factor, perhaps the maternal Ro(SSA) transplacentally passed to the infant, plays a role in the pathogenesis of the cutaneous and cardiac lesions of these neonatal lupus infants.

Immunogenetic studies indicate that the Ro(SSA) antibody is associated with both the HLA-DR2 and -DR3 phenotypes. The La(SSB) antibody, however, is found with an increased association with only the HLA-DR3 phenotype. This may explain why only the Ro(SSA) antibody has been found thus far in patients with the C2-deficient lupuslike disease (almost all, if not all, are of the HLA-DR2 phenotype).

Preliminary studies indicate that the Caucasian mothers of neonatal lupus infants are of the HLA-DR3 phenotype, but the infants demonstrate no association with a particular phenotype. This observation is potentially very important, because it strongly suggests that there is a linkage disequilibrium between the serologic reaction (Ro[SSA] antibody) but not the clinical expression of lupus.

CUTANEOUS MANIFESTATIONS OF COMPLEMENT DEFICIENCIES (See also Chapter 10.)

In recent years, individual deficiencies have been described for all of the 9 complement components. The isolated complement deficiencies are associated with a high incidence of associated cutaneous disease (Table 29–2). Absence of C1r, C1s, C2, C4, and C5 has been associated with the presence of cutaneous (discoid) as well as systemic lupus erythematosus. C2 deficiency, the most common inherited complement deficiency, has been found in normal individuals as well as in patients suffering from lupus erythematosus, anaphylactoid purpura, and dermatomyositis. C2 deficiency has been associated with an HLA haplotype consisting of A10 and B18.

Isolated deficiencies of the late complement sequence, ie, C3–C9, may also have prominent cutaneous features. A partial lipodystrophy has been described with low C3 concentrations (type II hypercatabolism). These patients have a C3 cleaving serum enzyme which is probably identical to the C3 nephritic factor described in patients with membranoproliferative glomerulonephritis. Several patients have now been described who have a partial lipodystrophy and mesangioproliferative glomerulonephritis with C3 nephritic factor in their serum. A single patient with a defect of C7 has been described. This individual appears to have the cutaneous and systemic findings of a mixed connective tissue syndrome.

Isolated deficiencies of properdin and C9 have recently been described. No systemic or cutaneous disease was detected in these patients.

It is interesting to note that a number of patients suffering from isolated deficiencies of various terminal complement components appear to have an increased susceptibility to repeated *Neisseria* infections. Six of 6 patients with a homozygous deficiency of C6, 5 of 10 with C7 deficiency, and 3 of 8 patients with C8 deficiency have had meningococcal or gonococcal bacteremia. In total, 16 of 30 patients with C6, C7, or C8 deficiency have had disease due to *Neisseria* bacteremia. In addition, 5 of 8 patients with homozygous C5 deficiency have had disease due to meningococcal

Table 29–2. Inherited and acquired deficiencies of the human complement system.

Component	Systemic Disease	Cutaneous Manifestations
C1q	Bacterial infections.	?
C1r	LE-like glomerulonephritis.	DLE-like lesions
C1s	SLE (not described), ANA-positive.	?
C4	LE-like syndrome, ANA-positive.	LE lesions
C2	SLE, glomerulonephritis, vasculitis, dermatomyositis. May be normal.	"Butterfly" rash, heliotrope, anaphylactoid purpura
C3	Recurrent pyogenic infections.	Pyoderma
C5	SLE. May be normal.	?
C6	Disseminated gonococcal disease.	Gonococcal cutaneous and arthritis syndromes
C7	Scleroderma. May be normal.	Scleroderma changes
C8	Disseminated gonococcal disease.	Xeroderma pigmentosum
C9	None.	None
$C\bar{1}s$ inhibitor ($C\bar{1}$ INH)	Hereditary angioneurotic edema, SLE, glomerulonephritis.	"Butterfly" rash, DLE lesions, angioedema
C3b inactivator (type 1 hypercatabolism of C3)	Recurrent pyogenic infections.	Pyoderma
Properdin	Fatal systemic infections	?
β_1 H	Hemolytic uremic syndrome	?
Acquired $C\bar{1}$ INH deficiency	Angioedema, paraproteinemias, lymphoproliferative disorders.	Angioedema, diffuse plane xanthomatosis
C3 hypercatabolism (type II hypercatabolism)	Recurrent pyogenic infections, nephritis.	Partial lipodystrophy
C5 dysfunction	Failure to thrive, recurrent gram-negative infections.	Leiner's syndrome
Opsonic defect in sickle cell disease	Pneumococcal meningitis, osteomyelitis.	None

LE = lupus erythematosus; SLE = systemic lupus erythematosus; DLE = discoid lupus erythematosus.

or gonococcal bacteremia. These studies indicate that patients with repeated *Neisseria* bacteremia are much more likely to have late-acting complement deficiencies.

Defects in the regulatory proteins of the complement system may be associated with prominent cutaneous manifestations. Patients born with an absence or dysfunction of C1 inactivator have hereditary angioneurotic edema. These patients may initially present to the dermatologist with an explosive onset of painless edema of the eyes and lips. Recently, several patients with hereditary angioneurotic edema have also developed SLE. At least one of the patients had the classic butterfly rash.

LICHEN MYXEDEMATOSUS (Papular Mucinosis)

This is a generalized dermal infiltrative process characterized by the presence of large amounts of dermal proteoglycans composed predominantly of the following glycosaminoglycans: hyaluronic acid, chondroitin sulfate, and heparan sulfate. A fibrotic variant, characterized histologically by fibroblastic proliferation and clinically by firm papules and plaques, is termed scleromyxedema. There is also evidence that fibroblasts cultured in vitro in the presence of serum from lichen myxedematosus patients—but not normal sera—undergo increased DNA synthesis and proliferation.

This dermal infiltrative process is associated with the consistent presence in the serum of a highly basic, electrophoretically slow IgG paraprotein. This unique paraprotein is almost always composed of λ L chains, but these paraproteins do not share idiotypic specificity with each other. The IgG λ paraprotein has a molecular weight of approximately 110,000, and the Fd portion of the γ heavy chain is partially or completely deleted. The exact relationship of the paraprotein to the cutaneous disease is unknown.

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30 Infectious Diseases

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Infectious diseases are associated so intimately with the functions of the immune system that it is possible to classify every human infection on the basis of local/systemic, specific/nonspecific, and cellular/humoral immune mechanisms. In essence, any infectious disease implies that the immune defense system has been successfully breached. It was the search for knowledge about protection against infection that yielded much of the basic information underlying the science of immunology today.

In this chapter, representative infectious diseases have been separated into categories based upon broad patterns of interaction between pathogenic microorganisms and the components of the immune system (Table 30–1). Such a classification is by nature arbitrary; clearly, such categories are not mutually exclusive. However, we hope this approach will emphasize some of the common immunologic features of diverse infective processes. Such an approach is not intended to serve as a substitute for the traditional study of specific pathogens and clinical syndromes.

EXTRACELLULAR INFECTIONS IN WHICH OPSONINS & POLYMORPHONUCLEAR NEUTROPHILS ARE DECISIVE IN RECOVERY

Major Immunologic Features

- Microorganisms possess antiphagocytic surface factors.
- Serum opsonins promote phagocytosis.
- Phagocytosis by polymorphonuclear neutrophils (PMNs) is followed by microbial death.
- Infection may progress because of qualitative or quantitative defects of opsonins or PMNs.
- Lymphocytes and macrophages apparently play no decisive role.

General Considerations

Many microorganisms are characterized by the presence of surface factors that retard phagocytosis. Since their presence in tissues stimulates an outpouring of PMNs, they are known as **pyogenic microorganisms.** Because they are highly susceptible to being killed by PMNs, they rely upon evasion of phagocytosis for their survival. Thus, they are also known as **extracellular pathogens.** Opsonins are humoral factors that promote phagocytosis and are needed to overcome antiphagocytic surface factors so that PMNs can ingest these microorganisms. Examples of microorganisms that must evade phagocytosis in order to survive (and the nature of the antiphagocytic surface factors) include *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Klebsiella pneumoniae, Escherichia coli, Bacteroides fragilis,* and *Pseudomonas aeruginosa*—especially cystic fibrosis strains (capsular polysaccharide); *Streptococcus pyogenes* (hyaluronic acid and M protein); *Staphylococcus aureus* (protein A); and *Neisseria gonorrhoeae* (pili composed of protein). Both *Yersinia pestis* and *Bacillus anthracis* possess antiphagocytic surface factors (Fra antigen and capsular polypeptides, respectively), but these may not play a primary role in disease pathogenesis.

1. *STREPTOCOCCUS PNEUMONIAE* INFECTION

S pneumoniae (pneumococcus) is a gram-positive lancet-shaped diplococcus that is found normally in the pharynx in 40–70% of adults. Infection is uncommon in comparison with the organism's frequent presence, generally occurring when there is a breach of host defenses such as aspiration, inhalation of irritants, viral upper respiratory infection, or pulmonary edema. An estimated 420,000 cases of pneumococcal pneumonia occur yearly in the USA, and pneumococci rank second to *H influenzae* as a cause of bacterial meningitis.

Pneumococci possess a series of 83 antigenically specific capsular polysaccharides that confer type-specific immunity in mice.

The capsules are composed of large polysaccharides which are hydrophilic gels. The complete structures of only a few capsular types (types 3, 6, and 8) are known. Type 3, for example, has a capsule composed of repeating cellobiuronic acid units joined by $\beta(1\rightarrow3)$ glucosidic bonds. Capsular polysaccharide subserves an antiphagocytic function. The in vivo opsonic effects of both IgM and IgG anticapsular antibody are mediated via their ability to activate complement. Although anti-cell wall IgG is capable of activating complement and fixing C3 to pneumococci, it is not opsonic—probably because C3 is deposited in sites on the pneumococcus that cannot interact efficiently with phagocytic cell C3 receptors. The mechanisms of host defense against encapsulated bacteria are discussed in Chapter 13.

Table 30–1. Infectious diseases classified by mechanisms of immunity, associated diseases, or causative agents.

Extracellular infections in which opsonins and PMNs are decisive in recovery: Infection due to–
Streptococcus pneumoniae
Streptococci of groups A and B
Staphylococcus aureus
Haemophilus influenzae
Neisseria meningitidis
Neisseria gonorrhoeae
Enteric gram-negative rods
Infections in which antibody may be decisive in prevention or in recovery through a mechanism other than opsonization: Diseases in which antibody–
Neutralizes exotoxins
Blocks epithelial attachment
Participates in complement-mediated bacteriolysis
Neutralizes viruses
Infections in which humoral and cell-mediated immunity collaborate in host defense:
Syphilis
Cryptococcosis
Candidiasis
Salmonellosis
Listeriosis
Intracellular infections in which lymphocytes and macrophages are decisive in recovery and humoral mechanisms play no protective role:
Measurement of antibody not useful in diagnosis and prognosis
Tuberculosis
Leprosy
Measurement of antibody useful in diagnosis and prognosis
Histoplasmosis
Coccidioidomycosis
Brucellosis
Tularemia
Infections characterized by unique host-parasite relationships:
Mycoplasma pneumoniae infection
Bordetella pertussis infection
Chlamydial infection
Rickettsial infection
Infections complicated by deposition of circulating immune complexes:
Infective endocarditis
Viral hepatitis
Poststreptococcal glomerulonephritis
Quartan malaria
Syphilis
Typhoid fever
Leprosy
The spectrum of host-virus immunologic relationships:
Viral diseases (acute, chronic, latent, slow)
Opportunistic infections: Infections associated with–
Hypogammaglobulinemia
Granulocytopenia
Depressed cellular immunity
Hemolytic anemia
Splenectomy
Foreign bodies
Gastrectomy

In general, the amount of capsular material is directly proportionate to the degree of virulence. Types 1, 2, 3, 5, 7, and 8 are all considered highly virulent. Type 3 pneumococci generally have the largest capsules and are the most difficult to phagocytize; infections with these microorganisms are associated with a poor prognosis. Type 3 pneumococci may result in pulmonary abscesses, which are extremely rare in infection with other types. There is some evidence that type 14 pneumococci share antigenic determinants with blood group substances. Such infections would theoretically be harder to control, since the host might have difficulty discerning "nonself" from "self."

Pneumococcal polysaccharide dissociates from the surface of microorganisms and may be detectable in the tissues, blood, and urine for some time after recovery from pneumococcal infection. There is evidence that this material may be endocytosed and later extruded from macrophages. A high level of pneumococcal polysaccharide antigenemia is associated with a less favorable prognosis for recovery from pneumococcal pneumonia.

Aside from the capsular antigens of pneumococci, other immunogenic constituents of the microorganisms (C substance, M protein, IgA1 protease, etc) play a less certain role in virulence or host response. However, C substance, a polysaccharide antigen probably equivalent to the group-specific C substances of *S pyogenes*, does have the peculiar ability to precipitate a β-globulin (C-reactive protein) found in the sera of patients with diverse inflammatory diseases. C-reactive protein binds and promotes the phagocytosis of a variety of bacteria by human PMNs. Among these are *S pneumoniae*, *S aureus*, and *E coli*. In addition, C-reactive protein and monoclonal antibodies directed against the phosphocholine component of C carbohydrate protect mice against otherwise fatal infection with *S pneumoniae*.

Clinical Features

Pneumococcal pneumonia begins classically with a single hard shaking chill, pleuritic chest pain, and cough productive of bloody (rusty) sputum. Bacteremia is a regular early feature of infection, generally occurring in close temporal relationship to the chill. The bacteremia may be self-limited or may result in metastatic infection of heart valves, meninges, or joints. Patients with ascites due to cirrhosis or the nephrotic syndrome seem particularly prone to pneumococcal peritonitis, often in the absence of any obvious respiratory infection. Prior to the advent of antimicrobial therapy, about two-thirds of patients with pneumococcal pneumonia would spontaneously recover by "crisis," the change in clinical course reflecting the synthesis of specific anticapsular antibody and the resultant enhancement of the phagocytic process after several days of acute illness.

Immunologic Diagnosis

Individual capsular types of pneumococci can be identified by the quellung phenomenon (Ger *Quellung*

swelling). In the presence of type-specific antiserum, polysaccharide capsules undergo refractive changes and swelling that can be detected by light microscopy, especially if the preparation is examined in the presence of India ink. In the era preceding the use of antimicrobial agents, precise identification of capsular type was very important; antisera used in treatment were often selected on the basis of the capsular type of the infecting strain. The capsular types most often associated with infection may vary from time to time and from community to community. Effective therapy with penicillin and other antibiotics has done away with the need for identifying individual capsular types except for epidemiologic purposes.

Some investigators employ a polyvalent omniserum to assist in rapid identification of pneumococci in clinical specimens. This reagent consists of a mixture of antisera to capsules of the common pneumococcal types.

The presence of antibody to a given pneumococcal capsular type can be detected by the Francis skin test. Here, capsular polysaccharide injected into the skin produces a wheal-and-flare response if antibody is present. This test is rarely if ever employed today. However, the response may be elicited unintentionally if a patient with a recent pneumococcal infection is immunized with pneumococcal polysaccharide vaccine containing a pertinent capsular serotype.

Pneumococcal capsular polysaccharide antigen can be detected in blood and other body fluids by counterelectrophoresis or latex particle agglutination techniques. Although blood cultures are generally positive when pneumococcal polysaccharide is detectable in the blood, the advantage of immunologic detection of this antigen is the rapidity with which diagnosis may be established (around 30 minutes) so that definitive treatment can be started. A full day may be required before blood cultures show evidence of growth (see Chapter 17).

Differential Diagnosis

Pneumococcal infection must be differentiated from other bacterial and viral pneumonias, from fungal and mycobacterial infections, and from noninfective processes such as pulmonary embolization. The diagnosis is established by isolation and cultivation of the infecting pathogen.

Prevention

Persons 50 years of age and older, patients with underlying immune defects (splenectomy; sickle cell anemia), and those with debilitating illnesses may be unusually susceptible to pneumococcal infection. These persons can usually be protected by active immunization with specific capsular types of pneumococci. Type-specific antibody to purified pneumococcal polysaccharide readily promotes phagocytosis of encapsulated pneumococci.

A 23-valent vaccine is currently used in the USA. Each dose contains 25 μg of polysaccharide from Danish types 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. The 23 types represented are responsible for 87% of the bacteremic pneumococcal disease reported to the Centers for Disease Control in 1983. Nasopharyngeal acquisition of pneumococcal types included in the vaccine appears to be reduced by vaccination. The duration of protection is as yet unknown, but elevated antibody levels persist for years, and booster immunizations are not currently recommended.

Antibody responses to pneumococcal vaccine in infants and very young children are not as reliable as in older persons. Types 6, 9, 14, 19, and 23 seem to be particularly poor immunogens in children below the age of 2 years.

Because the capsular polysaccharides of certain strains of *K pneumoniae* and *S pneumoniae* are quite similar, polyvalent pneumococcal vaccine may provide a minor degree of cross-protection against *Klebsiella* infections.

Treatment

Penicillin is the drug of choice in the treatment of pneumococcal pneumonia and its local and hematogenous complications. Recent reports of pneumococcal resistance to penicillin may make the issue of pneumococcal vaccination a more urgent one in the future.

Complications & Prognosis

A small percentage of patients with bacteremic pneumococcal pneumonia die regardless of the rapidity with which a diagnosis is established and specific bactericidal antibiotic therapy is begun. The factors responsible for this presently "irreducible minimum" of deaths are uncertain, but it is this group that might be most benefited by prior pneumococcal vaccination. The mortality rate is higher in patients at the extremes of age; in alcoholics; in those with multilobar pulmonary involvement, meningitis, or endocarditis; in those infected with type 3 pneumococci; in those with profound leukocytopenia; and in patients who have been previously splenectomized. Splenectomy often portends a fulminating clinical course, with death in less than a day. There may be associated disseminated intravascular coagulation.

2. STREPTOCOCCI OF GROUPS A & B

The genus *Streptococcus* comprises a heterogeneous group of microorganisms with a broad range of animal hosts. They can be divided into a number of immunologically specific groups (A–O) based upon the presence of group-specific carbohydrate antigens in their cell walls. The group-specific carbohydrate of group A streptococci is N-acetylglucosamine. The most common etiologic agents of streptococcal infection in humans are from groups A, B, and D.

Group A Streptococcal Disease

Group A streptococci (with *S pyogenes* as the pro-

totype species) are the commonest streptococcal pathogens of humans, accounting for more (and more clinically distinct) disease than groups B and D. Group A strains produce complete (beta) hemolysis when streaked on sheep blood agar. Group B and D strains are usually nonhemolytic but may produce beta or alpha (incomplete, or "green") hemolysis.

Disease caused by *S pyogenes* may be suppurative (respiratory disease, impetigo, etc), toxigenic (scarlet fever; see Table 30–2), or nonsuppurative (acute rheumatic fever, acute glomerulonephritis; see Chapters 27 and 28).

Group A streptococci can be divided into 60 or more immunologic types based upon the presence of specific M proteins in the cell wall. Immunity to group A streptococci is type-specific; thus, several streptococcal infections can occur in the same person. Two other protein antigens (T and R) are not directly related to virulence but provide an important additional serologic scheme for subclassifying group A strains.

Group A streptococci possess 2 antiphagocytic surface components: **hyaluronic acid** (which is not immunogenic, presumably because of its close structural relationship to the ground substance of human connective tissue) and **M protein.** The M protein is a readily accessible surface antigen, not blocked by the hyaluronic acid envelope of encapsulated strains, and is displayed as surface fimbriae. M proteins are necessary for virulence. In nonimmune hosts, complement-fixing cell-surface structures are masked by fibrinogen bound to the surface of M protein. Consequently, recognition of virulent organisms requires complement-fixing antibodies directed against antigenic determinants of M protein that remain exposed after binding of fibrinogen. Non-M-typeable group A strains are avirulent and are phagocytized and killed in the absence of type-specific antibody. Although M protein was once considered the structure that mediated streptococcal attachment to epithelial surfaces, it is now known that this function is subserved by lipoteichoic acid, the epithelial cell binding site for which is fibronectin. A close structural relationship with tropomyosin, a component of the thin filament of muscle cells, may help to explain some of the immunologic cross-reactions between mammalian muscle and streptococcal components.

Group A streptococcal strains produce at least 20 distinct antigens, many of which have strong clinical diagnostic relevance. Streptolysins are responsible for the beta hemolysis observed in sheep blood agar. Streptolysin S (serum-dependent, oxygen-stable) produces surface hemolysis by a nonenzymatic surface-active mechanism. Streptolysin S lyses a wide range of cells and is probably responsible for the leukotoxic property of group A streptococci whereby PMNs are killed after ingestion of streptococci. Streptolysin O (oxygen-labile) produces subsurface hemolysis by altering membrane sterols of the red blood cells. Except for streptolysin S, all known extracellular products of group A streptococci are antigenic. Antibodies directed against one or more of these antigens are useful markers of recent streptococcal infections but are not protective.

The most commonly measured antibody has been antistreptolysin O. Three weeks after streptococcal pharyngitis, 80% of children will have a 4-fold increase in titer. In contrast, patients with impetigo, even when this is associated with acute glomerulonephritis, will have a feeble or absent antistreptolysin O response. This finding is not due to absence of streptolysin O production. Instead, skin cholesterol binds streptolysin O and appears to prevent the enzyme from serving as an effective antigen.

The "streptozyme" test was devised to measure antibodies to streptolysin O, deoxyribonuclease B, hyaluronidase, nicotinamide deaminase, streptokinase, and other unspecified antigens with a single reagent: sheep erythrocytes coated with streptococcal "extracellular products." The current lack of standardization between different lots of the streptozyme reagent may limit the comparability of results of tests at different times and in different laboratories. In some cases (children under age 2 years; patients with acute glomerulonephritis), the streptozyme test may be insufficiently accurate; individual enzyme assays may have to be performed.

Antibodies to group A carbohydrate appear at about the same time as the antistreptolysin O and anti-DNase B titers but are less sensitive indicators of infection. The M proteins are poor antigens in humans; type-specific antibodies may not be detectable until 30–60 days after onset of infection. Their presence correlates positively with the duration of the convalescent carrier state. However, type-specific protective antibodies may persist in serum for many years, and a small booster dose of homologous M antigen may produce a rapid increase in antibody titer.

Notwithstanding the rich immunologic potential of this microorganism, it is possible that none of the diverse sequelae of streptococcal infection would be encountered if prompt phagocytosis and killing were to take place. Previous attempts to develop M protein vaccines have been thwarted by the occurrence of local inflammatory reactions and even acute rheumatic fever. Recent studies with a highly purified polypeptide vaccine prepared from streptococcal M24 protein show promise that previous problems with M protein vaccines may soon be overcome. One of the protective determinants has been identified in a peptide fragment composed of only 12 amino acid residues.

Group B Streptococcal Disease

For more than a decade, *Streptococcus agalactiae* (the group B streptococcus) has been recognized as a leading cause of neonatal sepsis. Common manifestations of illness include bacteremia, meningitis, and pneumonia. The pneumonia is more common in newborns than in infants over 10 days of age.

The principal human reservoir of group B streptococci is the female genital tract. Infants may become infected during birth or as a result of nosocomial spread in the nursery.

Human isolates of group B streptococci can be divided into 5 capsular serotypes (Ia, Ib, Ic, II, and III). Type III accounts for 60% of all group B streptococcal infections in neonates and infants. The core antigen of type III organisms is identical to that of type 14 pneumococcal polysaccharide except for the additional presence of sialic acid. The sialic acid plays an important role in the virulence of type III group B streptococci by blocking the activation of the alternative complement pathway. Serum that lacks antibody to type III antigen cannot opsonize type III streptococci. Low levels of antibody promote opsonophagocytic killing by the classic complement pathway, whereas higher levels of antibody are required to modify the surface conformation of the inactivator antigen sufficiently to allow activation of the alternative complement pathway. The classic complement pathway in the absence of antibody suffices for the phagocytosis of type Ia.

Recent studies suggest that infants who develop type III meningitis are those whose mothers lack antibody to this microorganism. Thus, immunization of adult women should be an effective means of preventing infant disease by transplacental passage of antibody. Preliminary studies indicate that a safe and immunogenic type III capsular polysaccharide vaccine can be developed.

Group D Streptococcal Disease

Group D streptococci (particularly enterococci) are encountered most frequently as causes of urinary tract infection or endocarditis in humans. There are currently no useful immunodiagnostic or immunopreventive measures relative to these illnesses.

3. *STAPHYLOCOCCUS AUREUS* INFECTION

S aureus is a gram-positive coccal microorganism that grows in grapelike clusters. Our knowledge of the immunopathology of this common microorganism is incomplete.

There is probably no other human pathogen that produces as many candidate virulence factors ("aggressins") as *S aureus*. Among the best-characterized are alpha toxin (one of 4 known hemolysins), coagulase, lipase, leukocidin, enterotoxin, exfoliatin, and protein A. Toxic shock syndrome is probably based on the production of pyrogenic exotoxin C, a form of enterotoxin F. Coagulase production and virulence are so closely (although probably coincidentally) linked that coagulase positivity is often considered to be synonymous with staphylococcal virulence. Enterotoxins and exfoliatin will be considered elsewhere in this chapter.

The cell walls of most *S aureus* strains are composed of teichoic acids (40% of cell wall weight), peptidoglycan (50%), and protein A (5%). Teichoic acids are charged polymers of ribitol phosphate linked to muramic acid residues of the peptidoglycan. They may serve as attachment ligands, allowing *S aureus* to adhere to mucosal receptor sites. The peptidoglycan component is a linear polymer composed of repeating β-1,4-linked N-acetylglucosamine and N-acetylmuramic acid. Recent studies suggest that peptidoglycan is the key cell wall component involved in staphylococcal opsonization and that it not only binds to IgG but is capable of activating both the classic and alternative complement pathways. Protein A is distributed evenly on the outermost layer of the cell wall of most *S aureus* strains. Protein A has the unique ability to bind to the Fc portion of IgG1, IgG2, and IgG4, leading to the production of "pseudoimmune complexes"; this phenomenon provides a powerful tool with which to investigate the biologic mechanisms of antigen-antibody reactions. Protein A can also bind to the Fc receptors on PMNs, thereby interfering with opsonization and phagocytosis. However, protein A is also a true antigen and reacts with the Fab portion of specific antibody.

Although some special staphylococcal strains will demonstrate capsule formation under highly defined in vitro conditions, staphylococci are not generally considered to possess antiphagocytic capsules. Only in experiments with encapsulated strains can serum antibody be shown to have a clear role in staphylococcal immunity.

Staphylococcal infection is highly destructive and produces prominent abscess formation. Most staphylococci appear to be killed once they are ingested by PMNs, although a few may survive under experimental conditions. Whether escape from PMN bactericidal activity is an important virulence mechanism in staphylococcal disease is presently uncertain. There is evidence that cell-mediated immunity may play a role in host defense against staphylococcal infection, although granuloma formation is distinctly unusual in this disease.

The detection of teichoic acid antibodies in the blood may occasionally be useful in estimating the duration and degree of antigenemia in patients with *S aureus* infection. Staphylococcal antigen has also been detected directly in the blood, pleural fluid, pericardial fluid, and cerebrospinal fluid by radioimmunoassay and counterimmunoelectrophoresis. Its detection is sufficiently infrequent that it has been suspected of being complexed with previously existing antistaphylococcal antibody. Detection of staphylococcal antigen currently serves no useful diagnostic purpose.

4. *HAEMOPHILUS INFLUENZAE* INFECTION

H influenzae is a small pleomorphic gram-negative rod with fastidious growth requirements. Six types of *H influenzae* (a–f) have been identified on the basis of capsular polysaccharides. Invasive infection with *H influenzae* (meningitis, arthritis, cellulitis, epiglottitis) is virtually always due to type b strains. However, otitis media generally results from nontypeable strains.

An alternative classification scheme based on outer membrane protein composition offers additional insights into the epidemiology of *H influenzae* infection. A 39,000-MW protein is of particular interest because of its antigenicity in humans, its exposed position in the cell wall, and its abundance among proteins of the outer membrane.

H influenzae is the most common cause of bacterial meningitis in the first few years of life and is responsible for many deaths as well as mental retardation. It is also extremely important as a cause of otitis media and epiglottitis. Until recently, *H influenzae* was considered predominantly a pathogen of children. Protective antibody acquired as a result of experience with this microorganism in childhood appeared to prevent infections later in life. However, it is now apparent that the common use of antibiotics in childhood infections may attenuate development of protective antibody titers. Thus, more frequent episodes of *H influenzae* pneumonia, bacteremia, and even meningitis are being encountered in adults.

The nasopharynx is considered to be the principal site for carriage and dissemination of *H influenzae* strains. Most strains are nontypeable, but up to 38% of children have had nasopharyngeal carriage experience with type b strains by 5 years of age. The precise mechanism by which *H influenzae* strains attach to nasopharyngeal mucosa is under investigation; pili may be involved. Although most *H influenzae* strains produce IgA1 protease, its role in disease pathogenesis is uncertain.

The capsular polysaccharides of *H influenzae* are considered to subserve an antiphagocytic function. The specific carbohydrates of types a, b, and c are polysugarphosphates; capsular polysaccharide of the clinically important type b strain is composed of polyribose ribosyl phosphate (PRP, PRRP). In immunologically mature humans, single injections of PRP elicit antibodies that are bactericidal, opsonic, and protective (in animal challenge).

There has been much debate about the relative importance of opsonization and phagocytosis versus antibody- and complement-mediated bacteriolysis of *H influenzae* in protection against type b disease. In 1933, Fothergill and Wright noted an inverse relationship between the bactericidal activity of blood for *H influenzae* and susceptibility to meningeal infection. The period of peak susceptibility between 6 months and 3 years of age was considered to represent the gap reflecting loss of transplacental bactericidal antibody on the one hand and acquisition of active immunity on the other. There is now serious question about whether direct bactericidal activity of blood (ie, antibody- and complement-mediated bacteriolysis) plays any real role in protection or whether it is more important as an in vitro indicator of immunity. The preponderance of data suggests that opsonic antibody, directed principally against PRP of type b strains (and to some extent against somatic antigens), is the principal protective system. However, strain heterogeneity among type b organisms has been suggested by differences in their susceptibility to antibody-mediated, complement-dependent serum bacteriolysis. These differences may be attributable to variations in outer membrane protein composition, the role of which in disease pathogenesis is under active investigation.

Because not all persons become nasopharyngeal carriers of type b *H influenzae*, it is not clear why hematogenous infection (meningitis, etc) is so rarely encountered beyond 4 or 5 years of age in the absence of an immunizing event. It has been suggested that immunity may take place through colonization of body surfaces with microorganisms possessing cross-reactive surface antigens. For example, certain strains of *E coli* and *S pneumoniae* stimulate production of antibody which cross-reacts with type b *H influenzae* strains.

Clinical Features

The principal clinical manifestations of *H influenzae* infection in children are meningitis and otitis media. Unencapsulated species are also found in the respiratory secretions of adults with chronic obstructive pulmonary disease and may be responsible for intermittent infective exacerbations of chronic bronchitis or frank pneumonia.

Immunologic Diagnosis

The measurement of antibodies to *H influenzae* is not of practical diagnostic importance. Circulating type b PRP capsular antigen can be detected by a variety of techniques including counterelectrophoresis and latex particle agglutination. Rapid diagnosis of *H influenzae* meningitis has been made possible by examination of cerebrospinal fluid by counterelectrophoresis.

Differential Diagnosis

H influenzae must be considered in the differential diagnosis of a variety of pyogenic infective processes but may usually be suspected on clinical grounds as a cause of otitis, meningitis, or epiglottitis in children of susceptible age.

Prevention

Despite the availability of potent antibiotics, *H influenzae* meningitis is still an important cause of illness and death in children. There are an estimated 10,000 cases of *H influenzae* meningitis yearly, with 400–500 deaths and 3000–5000 survivors who have residual central nervous system damage. A vaccine has recently been developed against type b *H influenzae* based upon immunization with purified PRP. Adults respond to such a vaccine with long-lived bactericidal and opsonic antibody production. Side effects are minimal. Unfortunately, children under age 2 years (and especially under age 6 months)—the population at risk—respond poorly to PRP vaccination in terms of antibody response. Attempts to resolve this dilemma (which also exists for other polysaccharide vaccines) have included linkage of polysaccharide to a protein molecule (to elicit the T cell involvement that

T cell-independent polysaccharides cannot), the use of immune adjuvants, and the development of vaccines based at least in part on key outer membrane proteins.

H influenzae infections are transmissible to close personal contacts, especially in households or day care centers. Antibiotic prophylaxis with rifampin is currently recommended for close personal contacts (especially children) of patients with documented *H influenzae* infection.

Treatment

The drug of choice for *H influenzae* infection depends upon local patterns of antibiotic resistance. With the increasing frequency of ampicillin resistance among *H influenzae* strains, a variety of other antibiotics are now being used.

5. *NEISSERIA MENINGITIDIS* INFECTION

N meningitidis (meningococcus) is a gram-negative diplococcus with fastidious growth requirements. Meningococci possess 8 group-specific capsular polysaccharides with antiphagocytic activity (A, B, C, X, Y, Z, 29E, and 135). These antigens are major determinants of virulence, since nonencapsulated strains are incapable of producing progressive disease. Groups A, B, and C have been the most clinically important of the meningococci to date. Group A antigen consists of N-acetyl-O-acetylmannosamine phosphate. The B and C antigens both consist of N-acetylneuraminic acid (sialic acid), which is partially O-acetylated in the C antigen. Meningococci belonging to groups A and C differ from other meningococci in that capsular swelling (quellung phenomenon) can be demonstrated with specific antisera. Groups A and C capsular polysaccharides induce specific IgG or IgM antibody formation, whereas purified group B polysaccharide stimulates a pure IgM response. Because group B polysaccharide is relatively nonimmunogenic, it has been postulated that group B meningococcal neuraminic acid is so similar to that present on host cell membranes that the microorganism is not recognized as foreign. Alternatively, host neuraminidase might break down group B capsular polysaccharide too rapidly to allow potent immunity to develop. Recent studies suggest a strong correlation between the presence of the Km(1) allotype and the immune response to meningococcal group B vaccine.

Meningococci also possess type-specific antigens based upon the composition of their outer membrane proteins. The precise number of serotypes is a function of the method used in their demonstration (10 types by serum bactericidal reaction, 10 by SDS-polyacrylamide gel electrophoresis [SDS-PAGE], 15 by double immunodiffusion, 18 by solid phase radioimmunoassay). Serotypes are important determinants of virulence, but even "virulent" serotypes are capable of producing only nasopharyngeal carriage in the absence of encapsulation. Thus, disease pathogenesis appears to be related to specific combinations of serogroups and serotypes. Serotypes are shared among serogroups and are independent of serogroup. Meningococcal groups B, C, Y, and 135 share several serotype determinants, and over 50% of infections produced by these organisms are attributable to serotype 2 (immunodiffusion method). Serotype 2 is not found among meningococcal groups A, X, Z, or 29E, perhaps because these organisms have in common an absence of sialic acid in their capsules. Group A meningococci contain very few protein serotypes, and the one predominant serotype found is distinct from those in groups B and C meningococci.

Meningococci also possess a lipopolysaccharide-endotoxin complex (8 serotypes) that is thought to play an important role in the fulminating course of acute meningococcemia.

Meningococci are transmitted by airborne droplets. Adherence to nasopharyngeal epithelium appears to be mediated by pili (for encapsulated strains) or by outer membrane proteins (for unencapsulated strains). In a nasopharyngeal organ culture model, meningococci with pili attach in greater numbers than unpiliated meningococci. Whereas attachment of piliated meningococci differs markedly among epithelial cells from different sites, nonpiliated meningococci attach to all cell types in equal but low numbers. Thus, pili appear to subserve a specific attachment role in the process of nasopharyngeal colonization. Once attached, meningococci are able to enter nonciliated epithelial cells by a process akin to that of *N gonorrhoeae* in uterine tube mucosa.

Meningococcal carriers rapidly develop elevated humoral antibody levels in response to capsular and noncapsular antigens. The antibody produced is both opsonic and bactericidal. Despite the prompt humoral immune response, the antibody produced has no effect on the nasopharyngeal carrier state. Although meningococci produce an IgA1 protease, the role of this enzyme in perpetuating the nasopharyngeal carriage state is speculative.

The factors that lead to benign nasopharyngeal carriage of encapsulated strains for some meningococcal contacts and rapidly progressive infection for the others are uncertain. However, the presence of circulating meningococcal antibody (specifically, antibody that participates with complement in direct meningococcal bacteriolysis in vitro) appears to be an important indicator of protection. Whether actual bacteriolysis occurs in vivo or whether opsonic antibody alone is important in protection under clinical circumstances is uncertain. However, the susceptibility to hematogenous neisserial infections of patients lacking complement components C6, C7, or C8 suggests that direct serum bacteriolysis may indeed play a direct role in protection against meningococcal infection. Antimeningococcal IgA antibody has been shown to block the bactericidal activity of serum for meningococci. This may be particularly critical in the average person without specific meningococcal anticapsu-

lar IgG antibody. In such persons, "natural" serum bactericidal activity depends upon IgM antibody directed against meningococcal lipopolysaccharide. IgM-mediated bacteriolysis is blocked with particular facility by IgA.

Protective serum antibody may be stimulated in a number of ways: (1) nonencapsulated, nonvirulent meningococcal strains may colonize the nasopharynx and stimulate protection via antibodies to serotype antigens shared with encapsulated strains; (2) colonization by encapsulated strains of low virulence (such as group B serotypes 4 and 6) may elicit anticapsular antibody without producing disease; and (3) some *E coli* and *Bacillus* species possess capsular polysaccharides closely related to those of meningococci and may be responsible for natural immunity to *N meningitidis*.

Factors determining the occurrence of epidemics of meningococcal disease are not fully understood. However, it is clear that overall group-specific carrier rates are not the important determinant. What is important in the prediction of meningococcal outbreaks is information on strain-specific acquisition rates. Identification of strains requires determination of serogroup, serotype, and SDS-PAGE type (the last of these being useful in characterization of otherwise nontypeable strains).

Clinical Features

Meningococcal infection arising from the nasopharynx (or possibly from meningococci aspirated into the lungs) may produce a spectrum of clinical manifestations ranging from transient asymptomatic bacteremia to fulminating and rapidly fatal septicemia characterized by disseminated intravascular coagulation or Waterhouse-Friderichsen syndrome. Metastatic infection may involve joints, heart valves, and a wide variety of other loci, but the most common targets are the skin (infective vasculitis) and the meninges.

The meningococcal lipopolysaccharide-endotoxin complex may be responsible for the fulminating nature of meningococcemia and the production of disseminated intravascular coagulation, peripheral vascular collapse, and shock. Meningococci are known to "shed" endotoxin blebs in vitro, and they presumably do the same thing in vivo.

The rash of meningococcemia is typically widespread and purpuric. **Chronic meningococcemia** is a rare manifestation of meningococcal infection characterized by episodes of fever of a few days' duration recurring at daily, weekly, or monthly intervals. Rash is uncommon, but the occurrence of erythema nodosum-like lesions around the joints suggests that this disease may be partially due to the deposition of circulating immune complexes.

Immunologic Diagnosis

The measurement of antibodies to meningococci is not of practical diagnostic importance. However, the measurement of free capsular polysaccharide antigen by counterelectrophoresis and other techniques may have practical significance in rapidly establishing the diagnosis and prognosis of patients with meningococcal disease. In studies of group C meningococcal infection, the presence of meningococcal antigen in serum portended a severe clinical course; pretreatment levels were directly related to the degree of subsequent leukopenia, thrombocytopenia, and hypofibrinogenemia. High levels of antigen in the cerebrospinal fluid were associated with prolonged coma and elevated intracranial pressure.

Differential Diagnosis

A variety of pyogenic microorganisms (particularly pneumococci and *H influenzae*) can produce purulent meningitis. Skin rash and the occurrence of disseminated intravascular coagulation are suggestive but not diagnostic of meningococcal infection. The diagnosis is established by isolating *N meningitidis* on appropriate bacteriologic media.

Prevention

Until 10 years ago, the danger of meningococcal spread could be minimized by the simple expedient of treating close personal contacts of patients and carriers with prophylactic sulfonamides. The advent of sulfonamide-resistant meningococci prompted a search for alternative chemoprophylactic agents, none of which have been fully satisfactory. At present, rifampin is the drug of choice for meningococcal prophylaxis.

Three meningococcal vaccines composed of purified meningococcal polysaccharide (monovalent A, monovalent C, and bivalent A and C vaccine) are available for use. In 1982, a quadrivalent vaccine, also containing W135 and Y strain antigens, became available and was recommended for military use. These vaccines are highly effective in preventing meningococcal infection; side effects are negligible. The vaccines induce protective opsonic and bactericidal antibodies. It has been suggested that vaccination should be considered an adjunct to antibiotic chemoprophylaxis for household contacts of patients with meningococcal disease, since half of the secondary family cases occur more than 5 days after the onset of the primary case; this is considered long enough to yield potential benefit from vaccination in case antibiotic prophylaxis is not successful.

There remain a number of problems with meningococcal vaccines. Of major concern is the apparent lack of immunogenicity of group B polysaccharides and the poor antibody responses of infants and young children to group C polysaccharides.

The nonimmunogenicity of the purified group B polysaccharide has caused a renewed interest in the vaccine potential of major outer membrane proteins of *N meningitidis*, especially serotype 2. Although purified type 2 outer membrane protein is poorly immunogenic when given alone, recent studies involving immunization of a small number of volunteers with a noncovalent complex of meningococcal group B polysaccharide and type 2 outer membrane proteins

demonstrate that both components of the complex are immunogenic when presented in this form.

Another approach to vaccination is suggested by the fact that the K1 capsular polysaccharide antigen of *E coli* is practically identical to that of group B meningococci. (More than 80% of neonatal meningitis cases attributable to *E coli* possess the K1 antigen.) Antibody is formed to the K1 antigen in experimental animals.

Treatment

Meningococcal meningitis and other complications of meningococcemia are best treated with penicillin. Because disseminated intravascular coagulation often complicates meningococcemia, anticoagulation with heparin has been suggested as a therapeutic adjunct. This cannot currently be considered of definite benefit.

Complications & Prognosis

Acute meningococcemia carries a high mortality rate, and meningococcal meningitis may be followed by neurologic defects and impaired learning. Speed in establishing the diagnosis and initiating specific therapy is essential. There is virtually no other acute infectious disease that can kill with the rapidity of meningococcemia. The occurrence of meningitis is, paradoxically, a good prognostic sign, since it indicates that the patient has survived the initial bacteremia long enough to develop symptomatic secondary metastatic infection.

The occurrence of bilateral adrenal hemorrhage during acute meningococcemia (as part of the Waterhouse-Friderichsen syndrome) has often prompted the use of corticosteroid therapy on the grounds that death occurs from acute adrenal insufficiency. In fact, direct measurements of adrenal corticosteroids in the blood indicate no such deficiency. Adrenal hemorrhage presumably reflects the general occurrence of vasculitis and disseminated intravascular coagulation.

6. *NEISSERIA GONORRHOEAE* INFECTION

N gonorrhoeae (gonococcus) is a gram-negative diplococcus with fastidious growth requirements. Although tentative typing schemes based on nutritional requirements (auxotype) or composition of lipopolysaccharide, outer membrane protein, or pili have been proposed, none are in general use at this time.

Gonorrhea is currently epidemic throughout the world, with an estimated 100 million new cases occurring yearly. Factors predisposing to this epidemic include a short incubation period, high infectivity, widespread asymptomatic carriage (at least 80% of uterine cervical infections; up to 40% of male urethral infections; and perhaps the majority of pharyngeal and rectal infections in both sexes are asymptomatic), transmissibility of infection from asymptomatic carriage sites, absence of a reliable serologic test for infection, relaxed sexual mores, and greater resistance of current strains of gonococci to antimicrobial agents.

The upsurge in gonorrhea has rekindled interest in the immunologic pathogenesis of this disease, since it is now apparent that bactericidal antibiotics have not been the key to control.

The immunologic armamentarium of the gonococcus is only beginning to be appreciated. **Capsules** are often demonstrable but are poorly adherent; their role in pathogenesis is obscure. **Pili** are present on virulent gonococci and appear to play a role in epithelial cell attachment and in retarding ingestion by professional phagocytes. They may also attach to motile spermatozoa. In gonococcal outer membranes, 2 protein species predominate. **Protein I** (POMP, MOMP, protein 1) occurs in various subunit molecular-weight forms in different strains, is constant in subunit molecular weight among intrastrain variants, seems to be present in all gonococci, is exposed on the gonococcal surface, and probably has porin function (ie, forms pores that allow the passage of small water-soluble molecules through the outer membrane). **Protein II** (opaque protein, LA protein, hmP) comprises a family of surface-expressed protein species that are not present in all gonococci but are prominent in certain opaque colony forms. They may mediate attachment of gonococci to leukocytes (LA protein) as well as to epithelial cells and to one another.

The attachment of gonococci to epithelial surfaces is retarded by mucosal IgG and IgA. The role of gonococcal IgA1 protease in hastening mucosal colonization is speculative at this time. **Lipopolysaccharide** (endotoxin) released from dying gonococci attached to uterine tube epithelial cells has the capacity to cause adjacent ciliated epithelial cells to detach and slough. This may represent the mechanism by which uterine tubes are so severely damaged by gonococcal infection.

Viable attached gonococci are endocytosed by epithelial cells by an unknown mechanism and transported to the base of the cells. Within the epithelial cell they are protected from antibody, complement, and antibiotics. Rupture of epithelial cells often produces large submucosal vacuoles filled with gonococci. Presumably this process precedes the invasion of gonococci into the bloodstream.

Gonococci can generally be killed by the bacteriolytic activity of complement and specific antibody, the antibody being directed against lipopolysaccharide and protein I. However, gonococci that produce hematogenous infection (**disseminated gonococcal infection, DGI**) have the capacity to bind an IgG antibody to protein I that blocks serum bactericidal activity. DGI strains of gonococci also have other unique attributes including the AHU (arginine-hypoxanthine-uracil)-requiring auxotype, high susceptibility to penicillin, and reduced ability to activate the alternative complement pathway (thus resulting in a minimal inflammatory response at infected mucosal sites). They also lack protein II and thus form transparent, rather than opaque, colonies.

In general, gonococci that are phagocytized by polymorphonuclear leukocytes are considered to be rapidly killed. However, experimental data relating to this question are contradictory, and the extent to which intracellular gonococci are killed remains uncertain. Serum antibody against *N gonorrhoeae* may be both opsonic and bactericidal. Patients with deficiencies of complement components C6, C7, or C8 appear to be at especially increased risk for hematogenous gonococcal infection.

It is noteworthy that seminal plasma, the milieu in which gonococci are normally transported, interferes with the progress of normal immune responses including the bacteriolytic activity of antibody and complement. Transferrin competes for the iron-binding siderophore of gonococci (gonobactin), thus depriving the microorganism of needed iron in body fluids in which transferrin is present.

Clinical Features

The principal clinical manifestations of gonorrhea are urethral exudate (males) and vaginal discharge. However, as noted above, mucosal carriage is asymptomatic in most sites in the majority of cases.

In approximately 1% of patients, gonococci gain access to the bloodstream and produce the syndrome of disseminated gonococcal infection, characterized by suppurative arthritis (often monoarticular), tenosynovitis, and metastatic skin lesions reflecting a frank infective vasculitis.

Immunologic Diagnosis

There currently is no reliable serologic test for gonorrhea. Attempts to develop such tests have been frustrated by the use of impure antigens and by unreproducible tests based on detection of small differences in antibody levels between patients and controls. The gonococcal complement fixation test and a more recent test based upon the agglutination of latex particles coated with crude gonococcal antigen lack apparent specificity and sensitivity. The development of techniques for detecting antibody to gonococcal pili holds promise that a means may become available for the detection of asymptomatic carriers, especially women, in whom antibody titers tend to be relatively high. At present, the most useful immunologic test for clinical purposes is probably detection of gonococcal antigens in skin lesions by immunofluorescence.

Differential Diagnosis

Gonococcal urethritis is easily diagnosed in males by the presence of typical gonococci in Gram-stained smears of urethral exudate. Nongonococcal urethritis (or nonspecific urethritis) also produces purulent urethral discharge. About half of these infections are due to *Chlamydia trachomatis* types D–K. Bacterial culture techniques remain the most reliable method for establishing a diagnosis of gonorrhea.

Prevention

Although there are significant cellular, humoral, and mucosal immune responses to infection with *N gonorrhoeae*, gonorrhea can occur repeatedly in sexually active populations. Lack of apparent solid immunity may be based on the likelihood that the immune response is specific to the infecting microorganism and that subsequent infections occur with gonococci of differing "serotype." Indeed, there is great diversity of cell surface antigens among various strains of gonococci: over 50 antigenically distinct types of pili; 16 type-specific outer membrane proteins; 4 distinct polysaccharides; and at least 5 or 6 distinct lipopolysaccharides. Furthermore, the precise chemical composition of the gonococcal capsule and the numbers of antigenically distinct capsular types are not yet known.

Development of gonococcal vaccine is considered to be of high priority, and 2 major structural components are under active investigation as potential immunogens: pili and principal outer membrane protein (POMP). Pilus-mediated attachment of gonococci to epithelial cells is inhibited by antibodies to pili, and maximal inhibition occurs when antibodies are directed to pili antigenically identical to those mediating attachment. In recent clinical trials, pilus vaccine has stimulated both mucosal and opsonic antibody with specificity for the immunizing strain. A pilus-based vaccine would seem to provide the best hope for preventing mucosal infection. A major problem yet to be overcome is the extraordinary antigenic diversity among pili. There is evidence that pili possess regions of common functional conformation. Perhaps advantage can be taken of this phenomenon so that development of a broadly reactive vaccine will be possible.

In recent studies in guinea pigs, isolated POMP complex proved to be a better protective immunogen than pili from the same gonococcal strains. POMP appears to react in antibody-complement-mediated killing of gonococci, whereas antibodies to pili are only weakly bactericidal. Protective immunity to gonococcal infection in the chimpanzee correlates best with bactericidal antibody in the serum of vaccinated chimpanzees. Thus, POMP also appears promising as an immunogen adaptable to a vaccine, with the advantage of stimulating bactericidal antibodies. Such a vaccine might be of value in the prevention of DGI. However, care must be taken that candidate POMP vaccines are incapable of stimulating the production of IgG-blocking antibody.

Treatment

Penicillin, tetracycline, and spectinomycin are the antibiotics most commonly used to treat all forms of gonorrhea.

Complications & Prognosis

The most serious complication of untreated gonococcal infection is salpingitis—a major cause of involuntary sterility, especially in developing countries. Disseminated gonococcal infection complicates approximately 1% of local mucosal infections and has been discussed above.

7. GRAM-NEGATIVE RODS (Enteric & Environmental)

There is one episode of gram-negative bacteremia for every 100 hospital admissions in the USA today. Antibiotics have failed to alter this situation in a meaningful way; 30–60% of patients with gram-negative sepsis continue to die despite antibiotic therapy—perhaps because of irreversible effects of endotoxin.

Immunization is successful for the prevention of disease due to exotoxin-producing bacteria or infections caused by certain specific serologic types of infecting microorganisms. In the case of the common hospital-acquired gram-negative rod infection, however, the situation is more complex; multiple species and serotypes of Enterobacteriaceae and Pseudomonadaceae are commonly involved. For example, 103 K, 164 O, and 75 H antigen types are recognized in *E coli*, and 80 capsular types of *Klebsiella* are known. As a result, immunization based upon type-specific antigens does not appear to be a realistic goal.

Because the core portions of lipopolysaccharide from most of these diverse microorganisms are of nearly identical chemical constitution, the possibility has been raised that antibody to rough mutants might be protective against infections caused by heterologous gram-negative bacilli. Support for this concept comes from studies that have shown an improved prognosis for survival in patients with gram-negative septicemia who possess high hemagglutinating antibody titers to core glycolipid. An understanding of the surface antigens of enteric bacilli and of the composition of bacterial lipopolysaccharide may be gained by examination of Figs 30–1 and 30–2, respectively.

Rough (R) strains are mutants that are blocked in biosynthesis of the complete O antigen (region I). There can also be mutations of the core polysaccharide (region II). Lipid A (region III) serves as the primer or membrane carrier upon which core polysaccharide is built. Ketodeoxyoctonate (KDO), one of the core sugars of region 2, is unique to bacterial lipopolysaccharide and links the core to lipid A. In the biosynthesis of the core region, each sugar is added by a specific enzyme. Hence, the potential exists for a series of core mutants, each dependent upon the absence of a specific enzyme. Ra mutants contain the complete core, whereas Rb through Rd mutants are deficient in one or more basal sugars. The Re mutant of lipopolysaccharide is composed solely of KDO and lipid A; these "extreme rough" mutants have the most incomplete lipopolysaccharide compatible with bacterial viability.

Antisera prepared from animals immunized with the Re mutant of *Salmonella minnesota* are able to provide passive protection to mice with *Klebsiella pneumoniae* bacteremia. The J5 mutant (Heath) of *E coli* O111 has been impressive in active and passive immunization studies involving both heterologous Enterobacteriaceae and Pseudomonadaceae. (J5 lacks the enzyme uridine diphosphate glucose 4-epimerase and therefore produces an incomplete lipopolysaccharide containing only lipid A, KDO, heptose, and glucose—a composition equivalent to that of the Rc mutants of *Salmonella*.)

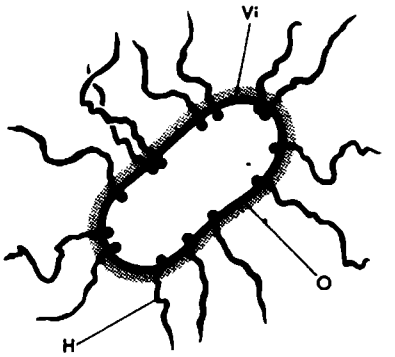

Figure 30–1. Schematic diagram of cellular locations of H, O, and Vi antigens of enteric bacilli. H=flagellar antigens (Ger *Hauch* breath; named for the flagellar-induced swarming of *Proteus* species on agar, which resembles the appearance of a breath on cold glass). O=lipopolysaccharide somatic antigen (Ger *ohne* without, ie, without flagella). Vi=an additional surface polysaccharide antigen of *S typhi* originally thought to be responsible for virulence. It is probably a special example of a K (Ger *Kapsel* capsule) antigen which is itself too thin to be seen as a capsule. (Reproduced, with permission, from Davis BD et al: *Microbiology*, 2nd ed. Harper & Row, 1973.)

The mechanism of protection of core antibody appears to involve both antiendotoxic and direct opsonic effects. Even in patients without circulating granulocytes, core antisera may be protective by enhancing phagocytosis of blood-borne gram-negative bacilli by the fixed mononuclear phagocytes of the liver and other reticuloendothelial tissues.

Studies with core glycolipid provide hope that immunoprophylactic and immunotherapeutic approaches may be of value in the management of nosocomial gram-negative rod infection. Alternative approaches involving pili and porins are under active investigation.

P aeruginosa is a major cause of nosocomial infection, and immunologic methods for prevention and

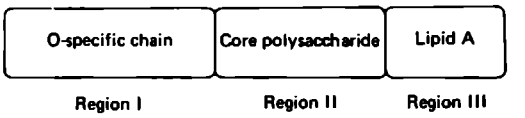

Figure 30–2. Structural diagram of bacterial lipopolysaccharides. Lipopolysaccharide (endotoxin) is composed of O-specific side chains consisting of repeating (oligosaccharide) units attached to a basal core polysaccharide which is attached in turn to lipid A. Rough mutants are blocked in biosynthesis of the complete O antigen. (Reproduced, with permission, from Davis BD et al: *Microbiology*, 2nd ed. Harper & Row, 1973.)

therapy have received considerable attention. PEV-01, a 16-part polyvalent cell surface extract, has been shown to have value in both active and passive immunization trials in burn patients in India. However, pili, cell-surface proteins, lipopolysaccharide, elastase, and proteases have also shown efficacy as vaccine components in various animal models of *P aeruginosa* infection. Both human IgG and α_2-macroglobulin (a protease inhibitor) have demonstrated protection in a mouse burn-wound model of *P aeruginosa* infection. Toxin A, a *P aeruginosa* exotoxin, is an important virulence factor in *Pseudomonas* infection. In an experimental model of burn-wound sepsis, the passive transfer of antitoxin or the active immunization with toxoid resulted in protection from live challenge. Whether immunotherapy directed at exotoxin A will improve therapeutic outcome in patients is controversial and awaits clinical trial.

8. PLAGUE

Plague is caused by a gram-negative rod *(Y pestis)*. Its pathophysiology and immunology are somewhat unique: It behaves in some respects as a facultative intracellular pathogen (multiplication in macrophages) and in others as an extracellular pathogen (killed by PMNs in the presence of opsonic antibody).

Plague is a natural disease of rodents and is transmitted by the bites of fleas. In the fleas, bacilli proliferate in the intestinal tract and produce obstruction. When the fleas next bite, they regurgitate bacilli and aspirated blood into the new host. If rodents are not available as hosts, humans may become involved by default. Bacilli enter dermal lymphatics and produce severe regional lymphadenitis (buboes; *bubonic* plague). Infection may disseminate hematogenously. When metastatic pneumonia occurs, infection may be spread by airborne droplets (*pneumonic* plague). This is a particularly contagious and malignant form of infection.

Bacilli contained in the gut of the flea have no antiphagocytic surface factors. Consequently, they are promptly ingested and destroyed by PMNs and monocytes. When ingested by macrophages, however, they survive, multiply, and emerge containing 2 new antigens: a capsular glycoprotein antigen (fraction 1; F1; Fra^+) and the Vwa^+ antigen system consisting of protein (V) and a lipoprotein (w). Apparently the low body temperature of the flea (25 °C) blocks development of these virulence factors. Other important virulence factors in *Y pestis* include the ability to synthesize endogenous purines (Pur^+), the bacteriocin pesticin plus linked invasive enzymes (Pst^+), a pigment-binding surface component (Pgm^+), and murine toxin (associated with the presence of a cryptic plasmid). Of all of these virulence factors, mutational loss of Pur, Pgm, and Vwa is associated with the most profound loss of virulence. Effective plague vaccines must contain Fra and Vwa moieties. The current vaccine consists of formalin-killed whole cells.

9. ANTHRAX

B anthracis is a large gram-positive, spore-forming microorganism possessing an antiphagocytic capsule composed of a gamma polypeptide of D-glutamic acid. Although important in the initiation of infection, evasion of phagocytosis is a less critical pathogenetic factor than toxin production once disease becomes established. Hence, virulence of the anthrax bacillus is due to both capsule formation and toxin production. Anticapsular antibody alone is not sufficient to prevent anthrax; vaccines must stimulate antitoxin immunity as well.

INFECTIONS IN WHICH ANTIBODY MAY BE DECISIVE IN PREVENTION OR IN RECOVERY THROUGH A MECHANISM OTHER THAN OPSONIZATION

1. DISEASES RESULTING FROM EXOTOXIN PRODUCTION (Table 30–2)

Major Immunologic Features

- Microorganisms are generally poorly invasive.
- Important disease manifestations are based predominantly upon toxin-related effects.
- Antitoxins may prevent the disease or ameliorate its course.

General Considerations

The diffusible toxins produced by certain gram-positive and gram-negative microorganisms are referred to as **exotoxins** because they are by-products of living bacteria and are not intrinsic to the bacterial cell walls (as are **endotoxins**). Exotoxins may be actively excreted by growing microorganisms in which no appreciable autolysis has occurred, or they may be released by microbial autolysis (intracellular toxins). All characterized exotoxins are proteins and thus are capable of stimulating antibody formation. In many of the diseases to be discussed, active immunization against exotoxins is carried out by preparing **toxoids** through the introduction of substituent groups such as formaldehyde or iodine. Toxoids are antigenic but essentially nontoxic. Theoretically, toxin genes can be specifically mutated in vitro, then exchanged for the wild type gene in the chromosome of the normal bacterial host. The result would be a "toxoid"-producing strain. This approach is under current investigation in the development of a recombinant cholera vaccine.

Many of the diseases in Table 30–2 rely solely upon toxin production for their clinical manifestations (eg, tetanus). In others, production of toxin is coincidental and not essential to disease production (eg, erythrogenic toxin elaborated by *S pyogenes*). In some diseases (eg, anthrax, pertussis, plague, *P aeruginosa* infection), exotoxins are suspected of playing important pathogenetic roles, but data are not sufficient to

Table 30–2. Diseases resulting from exotoxin production.

Bacterial Species	Occurrence in Nature	Disease Produced	Tissue Invasion	Toxin and Mechanisms of Action	Role of Antibody and Vaccines	Comments
Vibrio cholerae (gram-negative bacillus)	Human cases and carriers.	Cholera.	No, but intestinal mucosal attachment appears critical (via flagella? pili? other?).	Enterotoxin (choleragen), a chromosomal (ie, intrinsic) toxin. Toxin binds to G_{M1} gangliosides in target cell membrane → ADP ribosyl transferase activity with activation of adenylate cyclase → production of cAMP → hypersecretion of chloride, HCO_3^-, and water → severe diarrhea.	Presently unclear. Vaccines: (1) Parenteral killed bacterial vaccine: limited protection. (2) Parenteral toxoid: very limited protection. (3) Live attenuated oral vaccine still a research goal; should stimulate secretory IgA to block *V cholerae* attachment to bowel as well as stimulate local antitoxic activity in gut.	Noncholera vibrios (nonagglutinating [NAG] vibrios) have also been described that secrete enterotoxin and produce a similar clinical syndrome. ADP ribosyl transferase activation is a mechanism common to cholera, enterotoxigenic *E coli*, diphtheria, and *P aeruginosa* exotoxin A.
Escherichia coli (gram-negative bacillus)	Human cases and carriers; animals.	Diarrhea (enterotoxigenic *E coli* diarrhea).	No, but intestinal mucosal attachment via a piluslike colonization factor (Cf) appears critical.	Two enterotoxins (transmitted by extrachromosomal plasmids). Mechanism of heat-labile toxin (LT) similar to that of cholera toxin (G_{M1} ganglioside binding; ADP ribosyl transferase → cAMP, etc). Mechanism of heat-stable toxin (ST) involves activation of guanylate cyclase → cGMP. Since toxin production is plasmid-mediated, enterotoxin production is not serotype-specific.	Presently unclear. No available vaccines. Because of the transmissibility of plasmids among *E coli* of various serotypes, a vaccine prepared against *E coli* somatic antigens would be of little value. Vaccine directed against toxin or Cf of theoretic value.	Must be differentiated from invasive *E coli* syndrome that reflects actual epithelial invasion by *E coli* (enteropathogenic *E coli*). LT or ST activity (or both) has also been found in species of *Aeromonas, Enterobacter,* and *Klebsiella.*
Shigella dysenteriae type I (Shiga's bacillus) (gram-negative bacillus)	Human cases and carriers.	Dysentery.	Epithelial cells of gastrointestinal tract; rarely beyond submucosa.	Neurotoxin (producing motor ataxia in rabbits and meningismus in humans). May be identical to enterotoxin (produces diarrhea). Common mechanism of action seems to be capillary endothelial cell damage.	Presently unclear. No available vaccines. Parenteral toxoid immunization fails to prevent diarrhea in monkeys despite high antibody titers in serum.	Virulent *Shigella* species are both invasive and toxigenic. Stimulation of local gut immunity may be the key to protection.
Clostridium botulinum (gram-positive sporulating bacillus)	Soil. Rarely, gastrointestinal tract.	Botulism.	No.	Neurotoxin (released by cellular autolysis, ie, an intracellular toxin). Eight type-specific toxins known (A, B, C_1, C_2, D, E, F, G); only A, B, E, and F common in humans. Spores germinate in foods → ingestion of preformed toxin. Spores may rarely germinate in wounds. Toxin binds at neuromuscular junction; blocks presynaptic release of acetylcholine, resulting in impaired breathing and swallowing, diplopia, and flaccid paralysis.	No immunity conferred by infection; enough botulinus toxin to immunize is enough to kill. Active immunization: toxoid. Passive immunization: antitoxin (polyvalent A, B, E; equine); critical in treatment of botulism.	Botulism is less an infection than an intoxication. Botulinus toxin may enter the body in 3 ways: (1) preformed, in food (most common); (2) germination of spores in a contaminated wound; (3) germination of spores in the gastrointestinal tract (infants may present with "floppy infant" or "sudden infant death" syndrome).
Clostridium tetani (gram-positive sporulating bacillus)	Soil and gastrointestinal tract of humans and animals.	Tetanus.	No.	Tetanospasmin (released by cellular autolysis, ie, an intracellular toxin). Acts pre- or postsynaptically to block inhibition mediated by interneuronal neurons in the spinal cord → spasmodic muscle contractions (spastic paralysis). May also interfere with muscle relaxation.	No immunity conferred by infection. The lethal dose is insufficient to immunize. Active immunization: toxoid. Passive immunization: tetanus antitoxin (human).	Tetanolysin (a hemolysin) is also produced but plays no apparent role in neuromuscular problems.

Clostridium difficile (gram-positive sporulating bacillus)	Intestinal tracts of humans and animals; environment.	Diarrhea; pseudomembranous colitis.	No, but toxins produce focal and diffuse necrosis.	Cytotoxin (toxin B): direct cytotoxic effect on cell membranes of diverse cell lines; mechanism of action may involve stimulation of cGMP and suppression of cAMP. Enterotoxin (toxin A): causes fluid accumulation in rabbit ileal loop assay and death when injected intracecally in hamsters (neither occurs with toxin B).	No vaccine available. Cytotoxicity is blocked by *Clostridium sordellii* antitoxin (reversible cross-reaction) and *C difficile* antitoxin (specific).	The most common cause of diarrhea following antibiotic administration (especially clindamycin, ampicillin, cephalosporins). Diarrhea may be mild or severe; secretory and/or hemorrhagic; benign or fatal. Treatment: oral vancomycin or metronidazole (to kill *C difficile*) or anionic chelating agents (to bind toxins).
Clostridium perfringens (gram-positive sporulating bacillus)	Soil and gastrointestinal tract of humans and animals.	Gas gangrene (clostridial myonecrosis).	Minimal.	Eleven soluble toxins produced that may be active in various aspects of disease (including phospholipase C, collagenase, hemolysin, proteinase, and DNase). The chief lethal component is α toxin (phospholipase C; lecithinase). In some cases (especially clostridial myonecrosis of the uterus), sufficient α toxin may be liberated to produce massive intravascular hemolysis.	Highly questionable (although polyvalent equine antitoxin is often used in gas gangrene, even in the absence of the massive hemolysis syndrome). Commercially available antisera neutralize only a small portion of the spectrum of toxins.	*C novyi* and *C septicum* may also produce gas gangrene. Mainstays of treatment are surgical excision, antibiotics, and, probably, hyperbaric oxygen.
		Clostridial enterotoxin-mediated diarrhea.	No.	Enterotoxin: released by sporulating microorganisms in gastrointestinal tract which lyse to → toxin → diarrhea.	Systemic vaccination in animals without effect on toxin production in gut. No information in humans.	Perhaps due to ingestion of preformed toxin as well.
Corynebacterium diphtheriae (gram-positive bacillus)	Human cases and carriers.	Diphtheria.	No.	Diphtheritic toxin (produced only by corynebacteria which are themselves infected by a tox^+ temperate phage). Blocks protein synthesis by ADP ribosyl transferase-mediated binding of the ADP ribose moiety of NAD to elongation factor 2 (EF 2), an enzyme necessary for peptide chain elongation in protein synthesis. Toxin has diffuse effects but is principally manifested by cardiotoxicity (cardiomyopathy) and neurotoxicity (motor paralysis; cranial nerves generally first).	Antibody to toxin prevents toxin-related death but has no effect on epithelial cell attachment or production of pseudomembrane by *C diphtheriae*. Active immunization: toxoid. Passive immunization: equine antitoxin; critical in management of suspected diphtheria.	Can measure presence of circulating antitoxin by intradermal injection of diphtheria toxin (Schick test). Lack of response demonstrates immunity. Possibility of nonspecific reactivity requires multiple controls.

Table 30–2 (cont'd). Diseases resulting from exotoxin production.

Bacterial Species	Occurrence in Nature	Disease Produced	Tissue Invasion	Toxin and Mechanisms of Action	Role of Antibody and Vaccines	Comments
Streptococcus pyogenes (gram-positive coccus)	Human cases and carriers.	Scarlet fever.	Yes.	Erythrogenic toxin (produced by *S pyogenes* strains infected by a tox^+ temperate phage). Any *S pyogenes* can be converted to toxigenicity. Mechanism of action may be direct skin toxicity or hypersensitivity reaction. According to the latter theory, erythrogenic toxin is not the primary cause of the rash. Instead, this "pyrogenic exotoxin" sensitizes the skin to other products of the streptococcus.	Antibody can prevent rash but has no effect on streptococcal infection. Three immunologically distinct rash-producing toxins have been identified, explaining the occasional occurrence of several episodes of scarlet fever in the same patient.	An incidental complication of *S pyogenes* infection; clinically dramatic, but with no real pathogenic significance. Historically an important illness; not common in USA today. Can measure presence of circulating antitoxin by intradermal injection of erythrogenic toxin (Dick test). Lack of response demonstrates immunity. Can also use a skin test to establish diagnosis (Schultz-Charlton reaction). Here a scarlet fever rash is blanched by locally injected serum from a convalescent patient. Large numbers of other toxins are also produced by *S pyogenes* but without clear clinical syndromes.
Staphylococcus aureus (gram-positive coccus)	Human cases and carriers.	Scalded skin syndrome (Ritter's disease; toxic epidermal necrolysis; generalized exfoliative dermatitis; pemphigus neonatorum).	No.	Exfoliatin (often due to phage group II *S aureus*) produces disruption of desmosomes between granular cells of epidermis → epidermal cleavage plane. Intense erythema about mouth and nose spreads to neck, trunk, and extremities. Followed by loosening of epidermis, bulla formation, and peeling. Bullous impetigo and scarlatiniform rash (erythema without exfoliation) may be variants. More common in young children. Often no clear site of infection apparent.	Unclear. Immunization with exfoliatin will prevent its effects in mice.	Exfoliatin-positive strains appear unique in their ability to → subcutaneous infection in mice without the intervention of a foreign body. Since experimental skin infection with *S aureus* is ordinarily difficult to establish using bacteria alone, exfoliatin may be an important virulence factor.
		Staphylococcal food poisoning.	No.	Preformed enterotoxin (6 types) → vomiting and diarrhea (toxin absorbed → stimulation of vomiting center).	Unclear.	Ingestion of preformed toxin.
		Toxic shock syndrome.	No.	Candidate toxins include pyrogenic exotoxin C and enterotoxin F. The mechanism of toxicity is unknown.	Unknown.	Syndrome is most frequently encountered in women who use tampons and is associated with intravaginal colonization with *S aureus*. Manifestations include fever, hypotension, vomiting, diarrhea, and scarlatiniform rash with subsequent desquamation.

permit their inclusion in the table. Many fungi produce poisonous substances (mycotoxins). For example, aflatoxin from *Aspergillus flavus* may be important in hepatic neoplasia and may also impair antibody formation, complement activation, phagocytosis, and blastogenesis under diverse experimental circumstances. A discussion of mycotoxins is beyond the scope of this chapter.

2. INFECTIONS IN WHICH EPITHELIAL CELL ATTACHMENT IS THE CRITICAL FIRST STEP IN ESTABLISHMENT OF INFECTION

Secretory IgA as well as IgG may be critical in preventing the attachment to epithelial cells of potential pathogens (*Vibrio cholerae*, *Shigella* species, *Salmonella* species, etc). The physiologic role of secretory antibody and the activity of microbial IgA proteases are discussed elsewhere in this book (see Chapters 12 and 13).

3. COMPLEMENT-MEDIATED BACTERIOLYSIS

Many gram-negative microorganisms are lysed by complement in the presence of specific antibody. As already noted, measurement of such bactericidal antibody in meningococcal infections is an important means for assessing immunity to infection. Bactericidal antibody may play a protective role in gonococcal and meningococcal infection. There is considerable question, however, about whether this mechanism is operative in other infections. Part of the confusion arises because microorganisms that have been passaged in vitro are commonly used in tests of the serum bactericidal mechanism. Such microorganisms may be more susceptible to membrane damage than microorganisms encountered in vivo during actual infections. Further, patients with granulocytopenia do not appear to be protected from sepsis with antibody- and complement-susceptible gram-negative rods even when their humoral immune mechanisms are apparently intact.

Antibody- and complement-mediated bacteriolysis may be augmented by lysozyme. The practical significance of this interaction remains uncertain.

4. VIRAL NEUTRALIZATION

As noted in Chapter 13, direct inhibition of viral infectivity by interaction of antibody with viral surface antigens is a critically important host defense mechanism. Antibody prevents attachment and host cell penetration by susceptible viruses. Not all viruses are susceptible to the effects of antibody.

INFECTIONS IN WHICH HUMORAL & CELL-MEDIATED IMMUNITY COLLABORATE IN HOST DEFENSE

Major Immunologic Features

- An etiologically heterogeneous group.
- Infecting microorganisms may be extracellular or facultatively intracellular pathogens.
- The dominant protective immune mechanism varies with each pathogen.

General Considerations

The microorganisms in this group are extremely diverse. The justification for grouping them together at all is partially negative; they do not fit easily into the other groups. None of these microorganisms are considered to be either strictly extracellular (and under the control of opsonins and PMNs) or strictly intracellular (and under the sole control of lymphocytes and macrophages). Some of them are characterized by an apparent immunologic paradox: *Cryptococcus neoformans* possesses an antiphagocytic capsule, and yet PMNs do not appear to be the critical host defense; *Treponema pallidum* appears to function as an extracellular pathogen, and yet cell-mediated immunity is unquestionably important in its control.

1. SYPHILIS

T pallidum is a noncultivable, motile, highly infectious spirochete that appears to function predominantly as an extracellular pathogen. The organism attaches in vitro to receptor sites on host cells exclusively by its tapered ends, meanwhile retaining active motility. Ingestion by macrophages in vitro may be facilitated by the provision of specific antibody, but this point is unclear.

T pallidum is extremely susceptible to heat and drying, so that direct transfer by intimate contact, preferably in the presence of moisture, is essential for its survival. Sexual contact is therefore an ideal mode of transmission of syphilis. Syphilis occurs naturally only in humans.

Three clinically identifiable stages of syphilis are traditionally described. The first 2 (**primary** and **secondary** syphilis) both occur early in the infectious, spirochetemic stage of disease. The third stage (**tertiary** syphilis) occurs much later, following a period of prolonged latency, and reflects a tissue-damaging immunologic response to previously deposited microorganisms (bystander cell injury).

Syphilis is an unusual infection in that there is evidence for both humoral and cell-mediated immunity. However, the relative importance of each is unclear, and protective immunity against rechallenge is incomplete. Evidence for the participation of humoral immunity in syphilis is as follows:

(1) A variety of nonspecific ("reaginic") and

specific antibodies are regularly present in the serum of patients who have syphilis.

(2) *T pallidum*-immobilizing antibodies (TPIA) are regularly present in the serum of patients who have syphilis.

(3) The frequency with which TPIA are found increases as syphilis progresses to latent and tertiary infection.

(4) Partial immunity can be conferred in rabbits by passive transfer of serum from syphilis-immune animals. This protection is apparent when *T pallidum* is injected intracutaneously into rabbits. Chancres may be either prevented or delayed by passive immunization.

Interestingly, humans who have been experimentally infected with *T pallidum* also develop increased local resistance to rechallenge at a cutaneous site. This local resistance is referred to as **chancre immunity.** Chancre immunity persists if primary infection remains untreated and syphilis progresses to a latent stage. Although chancre immunity is indicative of heightened local resistance, it does not prevent the systemic spread of *T pallidum* from the site of initial challenge. Chancre immunity may be attributable to antibody, as both the immunity and reaginic antibodies wane after treatment of primary syphilis. The time required depends on the titer of antibody and the severity of the illness. For several reasons, it is not likely that the antibodies are completely protective:

(1) Treponemes from the initial infection persist during latent syphilis, even though there is resistance to a second challenge. This suggests that the organism has found sanctuary in some sort of privileged residence where it is immune from host defense.

(2) Some antibodies are nonspecific and are found in other diseases such as lupus erythematosus. They might even be directed against host rather than treponemal antigens.

(3) By preventing attachment of treponemes to cells in tissue culture, antibodies might in fact aid the organisms in escaping host defense mechanisms.

(4) Circulating immune complexes are formed during infection with *T pallidum*. They are demonstrable in sera of both rabbits and patients with syphilis. They may be composed of cardiolipin-anticardiolipin as well as of treponemal antigen-antitreponemal antibody. They may act to depress synthesis of IgG against independent antigens, such as sheep erythrocytes. It is conceivable that circulating immune complexes may prevent the host from synthesizing treponemicidal antibody during primary syphilis or from synthesizing antitreponemal antibody that might act in concert with cell-mediated immunity against *T pallidum*.

(5) The patterns of antibody production change during the course of untreated syphilis. Patients with secondary syphilis have antitreponemal antibody as well as anticardiolipin antibody. These antibodies are of both IgG and IgM classes. As the disease enters latency, antitreponemal IgM antibody production ceases and patients are left with antitreponemal IgG and anticardiolipin IgM and IgG. The clinical significance of this sequence of events is uncertain.

Recent investigations in animal models have increasingly implicated cell-mediated immunity as a critical element in host response to *T pallidum*. Evidence for the participation of cell-mediated immunity in syphilis is as follows:

(1) Passive transfer of syphilis immune serum is only partially protective and does not follow classic models of humoral immunity.

(2) Syphilis progresses through primary and secondary stages despite the presence of antibodies that immobilize the infecting organism.

(3) Delayed hypersensitivity to treponemal antigens is absent in primary and early secondary syphilis but develops late in secondary infection and is regularly present in latent and tertiary syphilis.

(4) Granulomatous lesions characterize tertiary syphilis.

(5) Immunization with killed microorganisms is usually unsuccessful, whereas immunization with live attenuated organisms has produced immunity.

(6) In vitro lymphocyte reactivity to treponemal and nontreponemal antigens and T lymphocyte counts are suppressed during primary and secondary syphilis.

(7) Infecting rabbits with *T pallidum* stimulates acquired cellular resistance to *Listeria;* this reaction is mediated by T lymphocytes.

It is puzzling why so much time is required for patients to develop humoral and cellular immunity to syphilis. One theory holds that the mucoid envelope of *T pallidum* renders it highly resistant to phagocytosis; only after the treponemes have remained in the host for some time is the mucoid coat broken down enough for phagocytosis to occur. (Treponemal mucopolysaccharides also suppress lymphocyte blastogenic response to Con A.) As a result, treponemal proliferation outstrips the rate of antigenic processing for stimulation of humoral and cellular immune mechanisms; a condition of "antigen overload" then occurs, with production of secondary immunosuppression. An alternative explanation is that sensitization with treponemal antigen leads primarily to generation of antibodies that then "block" antigenic sites, thereby inhibiting an appropriate cell-mediated immune response.

These proposed immunologic mechanisms are highly speculative.

Clinical Features

The first clinically apparent manifestation of syphilis (**primary** syphilis) is an indurated, circumscribed, relatively avascular and painless ulcer (**chancre**) at the site of treponemal inoculation. Spirochetemia with secondary metastatic distribution of microorganisms occurs within a few days after onset of local infection, but clinically apparent secondary lesions may not be observed for 2–4 weeks. The chancre lasts 10–14 days before healing spontaneously.

The presence of metastatic infection (**secondary** syphilis) is manifested by highly infectious mucocutaneous lesions of extraordinarily diverse description as well as headache, low-grade fever, diffuse lymphadenopathy, and a variety of more sporadic phenomena. The lesions of secondary syphilis ordinarily go on to apparent spontaneous resolution in the absence of treatment. However, until solid immunity develops—a matter of about 4 years—25% of untreated syphilitic patients may be susceptible to repeated episodes of spirochetemia and metastatic infection.

Following the resolution of secondary syphilis, the disease enters a period of **latency,** with only abnormal serologic tests to indicate the presence of infection. During this time, persistent or progressive focal infection is presumably taking place, but the precise site remains unknown in the absence of specific symptoms and signs. One site of potential latency, the central nervous system, can be evaluated by examining the cerebrospinal fluid, where pleocytosis, elevated protein, and a positive serologic test for syphilis are indicative of asymptomatic neurosyphilis.

Only about 15% of patients with untreated latent syphilis go on to develop symptomatic **tertiary** syphilis. Serious or fatal tertiary syphilis in adults is virtually limited to disease of the aorta (aortitis with aneurysm formation and secondary aortic valve insufficiency), the central nervous system (tabes dorsalis, general paresis), the eye (interstitial keratitis), or the ear (nerve deafness). Less frequently, the disease becomes apparent as localized single or multiple granulomas known as **gummas.** These lesions are typically found in skin, bones, liver, testes, or larynx. The histopathologic features of the gumma resemble those of earlier syphilitic lesions except that the vasculitis is associated with increased tissue necrosis and often frank caseation.

Immunologic Diagnosis

In its primary and secondary stages, syphilis is best diagnosed by darkfield microscopic examination of material from suspected lesions. Diagnostic serologic changes do not begin to occur until 14–21 days following acquisition of infection. Serologic tests provide important confirmatory evidence for secondary syphilis but are the only means of diagnosing latent infection. Many forms of tertiary syphilis can be suspected on clinical grounds, but serologic tests are important in confirming the diagnosis. Spirochetes are notoriously difficult to demonstrate in the late stages of syphilis.

Two main categories of serologic tests for syphilis (STS) are available: tests for reaginic antibody and tests for treponemal antibody.

A. Tests for Reaginic Antibody: (This is an unfortunate and confusing designation. There is no relationship between this antibody and IgE reaginic antibody.)

Patients with syphilis develop an antibody response to a tissue-derived substance (from beef heart) that is thought to be a component of mitochondrial membranes and has been called **cardiolipin.** Antibody to cardiolipin antigen is known as Wassermann, or reaginic, antibody. Numerous variations (and names) are associated with tests for this antigen. The simplest and most practical of these are the VDRL test (Venereal Disease Research Laboratory of USPHS), which employs a slide microflocculation technique and can provide qualitative and quantitative data, and the rapid plasma reagin (RPR) circle card test. Positive tests are considered to be diagnostic of syphilis when there is a high or increasing titer or when the medical history is compatible with primary or secondary syphilis. The tests may also be of prognostic aid in following response to therapy, since the antibody titer will revert to negative within 1 year of treatment of seropositive primary syphilis or within 2 years for secondary syphilis.

Biologic false-positive tests. Since cardiolipin antigen is found in the mitochondrial membranes of many mammalian tissues as well as in diverse microorganisms, it is not surprising that antibody to this antigen should appear in other diseases. A positive VDRL test may be encountered, for example, in infectious mononucleosis, leprosy, hepatitis, and systemic lupus erythematosus. Although the VDRL test lacks specificity for syphilis, its great sensitivity makes it extremely useful nonetheless.

B. Tests for Treponemal Antibody: The first test employed for detecting specific antitreponemal antibody was the *T pallidum* immobilization test (TPI). Although highly reliable, it proved to be too cumbersome for routine use. A major test employed until recently was the fluorescent *T pallidum* antibody test (FTA). If virulent *T pallidum* from an infected rabbit testicle is placed on a slide and overlaid with serum from a patient with antibody to treponemes, an antigen-antibody reaction will occur. The bound antibody can then be detected by means of a fluoresceinated anti-human γ-globulin antibody. Specificity of the test for *T pallidum* is enhanced by first absorbing the serum with nonpathogenic treponemal strains. This modification is referred to as the FTA-ABS test. (If specific anti-IgM antibody to human γ-globulin is employed, the acuteness of the infection or the occurrence of congenital syphilis can be assessed. However, this test may sometimes be falsely positive or negative in babies born of mothers with syphilis.)

The FTA-ABS test is reactive in approximately 80% of patients with primary syphilis (versus 50% for the VDRL test). Both tests are positive in virtually 100% of patients with secondary syphilis. Whereas the VDRL test shows a tendency to decline in titer with

successful treatment, the FTA-ABS test may remain positive for years. The FTA-ABS test is especially useful in confirming or ruling out a diagnosis of syphilis in patients with suspected biologic false-positive reactions to the VDRL test. However, even the FTA-ABS test may be susceptible to false positives, especially in the presence of lupus erythematosus.

The microhemagglutination-*T pallidum* (MHA-TP) test, a simple passive hemagglutination test, appears to be a satisfactory substitute for the FTA-ABS test. Its principal advantages are economy of technician time and money. Its results correlate closely with FTA-ABS except during primary and early secondary syphilis, when both the VDRL and FTA-ABS are more likely to show reactivity. The VDRL test is the only one that can be employed with reliability in the evaluation of cerebrospinal fluid.

The interpretation of serologic data in syphilis may be extremely complex in some cases. For example, a prozone phenomenon may be encountered in secondary syphilis; serofastness may characterize late syphilis; and the VDRL test may be negative in up to one-third of patients with late latent syphilis.

Differential Diagnosis

Syphilis produces sufficiently diverse clinical manifestations that a discussion of its differential diagnosis should be sought in a textbook of general internal medicine.

Prevention

Early treatment with antibiotics is the only way known to prevent the later ravages of syphilis.

Treatment

Penicillin is the drug of choice for syphilis in all its stages. Since the lesions of tertiary syphilis may be irreversible, it is crucial to identify and treat the disease before tertiary lesions begin.

Complications & Prognosis

The most frequent complication of treatment is the **Jarisch-Herxheimer reaction,** which occurs in up to half of patients with early syphilis and is manifested by fever, headache, myalgias, and exacerbation of cutaneous lesions. It has been suggested that this reaction may be mediated by IgE. The intensity of a Jarisch-Herxheimer reaction reflects the intensity of local inflammation prior to treatment and is thought to result from the release of antigenic material from dying microorganisms. The reaction is of short duration (2–4 hours) and generally not harmful, although shock and death have been attributed to this reaction in tertiary forms of the disease. (The Jarisch-Herxheimer reaction has also been described in the treatment of louse-borne borreliosis, brucellosis, and typhoid fever.)

Other immunologic complications of syphilis include paroxysmal cold hemoglobinuria and nephrotic syndrome.

In patients who fail to receive any treatment for syphilis, it is estimated that one in 13 will develop cardiovascular disease; one in 25 will become crippled or incapacitated; one in 44 will develop irreversible damage to the central nervous system; and one in 200 will become blind.

2. CRYPTOCOCCOSIS

C neoformans is a yeastlike fungus that reproduces by budding. A mycelial form, *Filobasidiella neoformans*, has recently been described. Parent and daughter cells are surrounded by a thick polysaccharide capsule, and a characteristic "halo" is produced by the capsule in the presence of India ink.

Humans are thought to acquire cryptococcosis from inhalation of fungi. Person-to-person transmission has not been documented. It is likely that many are exposed to *C neoformans*, but few develop the disease. There are an estimated 300 new cases of symptomatic cryptococcosis per year in the USA.

Among the major systemic mycoses, the interaction between the host and *C neoformans* is perhaps the least well understood. One problem in dissecting the immune response has been the absence of potent and specific antigens to use as immunologic tools. Poor antigenicity of *C neoformans* may also be a practical problem for the host during natural infection.

Once established in the tissues, cryptococci evoke 2 principal histopathologic patterns. In the first, fungi proliferate largely unchecked, forming large gelatinous masses of capsular polysaccharide surrounding clumps of yeasts. There is little tissue reaction, and no necrosis occurs. In the second, there is granuloma formation with macrophages, lymphocytes, and plasma cells. Cryptococci may be found in centrally located giant cells. Again, there is no necrosis. Reactive lymphadenopathy is not common in lymph nodes draining cryptococcal pulmonary lesions. Healing is not associated with the intense scarring and calcification that characterize histoplasmosis, tuberculosis, and other infections evoking an intense delayed hypersensitivity reaction (ie, there is no "bystander" tissue injury).

The most important pathogenic cryptococcal constituent identified thus far is capsular polysaccharide. Capsular polysaccharide contains a backbone of α-1,3-linked D-mannopyranoside residues. There are 4 serotypes of *C neoformans;* all are virulent. Capsular polysaccharide is poorly immunogenic, a problem that is reflected in the difficulties in immunizing laboratory animals and the low antibody titers in patients with cryptococcosis.

Capsular polysaccharide may function as an antiphagocytic material by covering up opsonic proteins that have bound to antigenic sites on the fungal surface, thus preventing their recognition by phagocytes. Even specific anticapsular antibody may be unable to potentiate phagocytosis if polysaccharide is present in excess, since opsonic antibody is extremely susceptible to neutralization by free polysaccharide. Nonen-

capsulated cryptococcal mutant strains that are easily phagocytized are avirulent for mice; the encapsulated parent strains are highly lethal. Addition of capsular polysaccharide to in vitro systems impairs phagocytosis by PMNs. In vivo inoculation of capsular polysaccharide shortens survival of mice when they are later challenged with encapsulated cryptococci.

Both antibody and complement appear to be important in potentiating phagocytosis of cryptococci. Guinea pigs and mice decomplemented in vivo have enhanced susceptibility to cryptococcosis. Mice of C5-deficient inbred strains are exquisitely susceptible to cryptococcosis. Complement and antibody may interact protectively in that only mice with normal complement activity can be passively immunized with high-titer rabbit anticryptococcal antibody. Complement activation appears to follow the alternative pathway.

Thus, it has been postulated that antibody- and complement-dependent opsonization may be the crucial factor in limiting cryptococcal infection in most tissues and that the prominence of central nervous system infection may represent an "escape" of fungi to a milieu in which complement components penetrate in low titer or not at all. However, this is speculative and deemphasizes the unquestionably important role of cell-mediated immune mechanisms in cryptococcosis. Without invoking an important role for lymphocytes and macrophages, it would be difficult to explain (1) the virtual absence of PMNs from nonneurologic lesions; (2) the classic granulomatous response present in many tissues; (3) the apparent lack of undue susceptibility of hypogammaglobulinemic patients to cryptococcosis; and (4) the susceptibility to cryptococcosis of patients with Hodgkin's disease.

There is now evidence that some polysaccharide- or protein-containing extracts prepared from cryptococci are able to elicit cell-mediated immune responses. The skin test response in sensitized humans and nonprimates is of the delayed type. Lymphocyte activation and MIF have been demonstrated in lymphocyte cultures from healthy subjects exposed to *C neoformans*. These responses appear to be defective in patients with active and even resolved cryptococcosis.

Animal models of infection also support a role for cell-mediated immunity. Immunization of mice with cryptococcal extracts elicits a delayed cutaneous hypersensitivity response that correlates with increased protection on challenge. Treatment with anti-mouse thymocyte globulin enhances susceptibility to cryptococcosis and ablates both the skin test reaction and protective effects of vaccination. The congenitally athymic (nude) mouse is exquisitely susceptible to cryptococcosis. Finally, activated macrophages have increased killing capacities against *C neoformans* and may be able to kill them by some mechanism other than phagocytosis.

From these bits of information, we are gradually gaining insight into the pathogenesis of cryptococcosis.

Clinical Features

The first encounter between humans and fungus is probably in the lung. In persons with intact immune defenses, cryptococci may reside saprophytically in the respiratory tract or produce pulmonary disease. In patients with sufficient immunity to develop symptomatic pulmonary cryptococcosis, clinically apparent dissemination to other sites is not common. However, with host immunosuppression, cryptococci can readily spread from a primary pulmonary focus.

Extrapulmonary dissemination accounts for 90% of all clinically apparent cases of cryptococcosis and is characteristically (although not exclusively) seen in patients with underlying immune deficiency. Half of these patients either have primary lymphoreticular disease or are receiving immunosuppressive medications for other disorders.

Without treatment, cryptococcal meningitis runs an irregular course of months to years, ending with seizures, hydrocephalus, dementia, coma, and death.

Immunologic Diagnosis

The diagnosis of cryptococcosis has traditionally depended upon the demonstration of microorganisms in cerebrospinal fluid by India ink staining, together with their isolation on appropriate artificial media. Attempts to establish a diagnosis of cryptococcosis on the basis of elevated antibody titers have been frustrated by the absence of reliable tests, reflecting perhaps the poor antigenicity of *C neoformans* and the fact that many infected patients have underlying immune disorders that might compromise antibody synthesis.

Unfortunately, some patients with cryptococcal meningitis have negative India ink preparations of the spinal fluid, negative cultures, and an absence of demonstrable antibody. In many of these patients, a diagnosis can be made using a recently developed test for free cryptococcal polysaccharide antigen. The test uses high-titer rabbit anticryptococcal antibody adsorbed onto latex beads. In the presence of even minute amounts of cryptococcal antigen, a suspension of the beads agglutinates. A positive test in either serum or cerebrospinal fluid is considered diagnostic of cryptococcosis. In addition to its diagnostic value, the latex cryptococcal agglutination test has prognostic value. Falling titers are associated with improvement, and many physicians use an end-titer of 1:4 or less to terminate therapy.

Tests for cell-mediated immunity are presently of little help, as "cryptococcin" is poorly defined, and many patients are anergic to skin test antigens during active disease. Some regain skin test reactivity as they improve, but others do not.

Prevention

There is no commercially available cryptococcal vaccine. Experimental models have failed to show a benefit from passive immunization with "immune" serum. On the other hand, prior infection of mice with low numbers of cryptococci appears to confer immu-

nity against later challenge with high numbers of the same strain. Immunization of mice with cryptococcal extracts or intact cells in Freund's complete adjuvant also provides protection. However, use of live vaccines would be hazardous to immunosuppressed humans, and Freund's complete adjuvant cannot be used in humans.

Treatment

The mainstay of treatment is amphotericin B given with or without flucytosine (5-fluorocytosine).

A few patients have been treated with transfer factor; however, the value of transfer factor in cryptococcosis remains controversial.

3. CANDIDIASIS
(See also Chapters 20 and 29.)

Candida species (especially *C albicans*) are acquired as mucosal and gastrointestinal tract commensals from the maternal vagina at birth. Delayed-type hypersensitivity and the presence of low levels of *Candida* antibody are characteristic of the normal, immune host. *C albicans* and other *Candida* species become pathogenic under circumstances that alter the anatomic, hormonal, microbial, or immunologic milieu—skin and nail infection (moisture, trauma); thrush, esophagitis (antibiotic therapy, AIDS); vaginitis (pregnancy, birth control pills); chronic mucocutaneous candidiasis syndrome (selective T cell abnormalities); mucosal and skin infections (diabetes mellitus); invasive hematogenous infection (neutropenia, leukemia, disruption of mucosal barriers, chronic intravascular access sites); and endocarditis (intravenous drug abuse, prosthetic heart valves).

Candida species colonize the mucous membranes by attaching to epithelial cells by a presumed polysaccharide (? mannan) ligand system. There is a hierarchy of adherence among *Candida* species, with *C albicans* and *Candida tropicalis* more adherent than the others (*Candida krusei, Candida parapsilosis, Candida pseudotropicalis, Candida (Torulopsis) glabrata,* etc), regardless of the surface to which the organisms attach (eg, epithelial cells, endothelium, valve tissue, fibrin-platelet aggregates, dental acrylic material, lucite).

The pathophysiology of invasive *Candida* infection (as opposed to colonization) is probably best understood for *C albicans*, the most common etiologic agent of the syndromes described above, and *C tropicalis*, an increasingly important opportunistic pathogen. These fungi colonize mucosal surfaces as yeasts that reproduce by the budding of a single daughter cell (blastospore). Under conditions of restricted nutrients (in vitro) or altered host status (in vivo), the fungi undergo filamentous transformation with the production of pseudohyphae (elongated blastospores, budding from one another in linear sequence, with characteristic constrictions at the site of budding) or true hyphae. There is some evidence that yeasts can initiate the process of tissue invasion by the mediation of phospholipases but that filamentous transformation completes the process. There are antigenic differences between yeasts and filamentous forms. Germinated forms of *C albicans* adhere more avidly to mucosal surfaces than yeasts.

Nonspecific host defense mechanisms operative against *Candida* infection include normal flora (bacterial interference); iron restriction (transferrin, lactoferrin); epithelial cell turnover; washing and enzymatic (lysozyme, lactoperoxidase) activity of mucosal secretions; and baseline phagocytic activities. The role of SIgA in preventing *Candida* adherence is uncertain.

Phagocytosis can be potentiated by either the classic or alternative complement pathway, and the only apparent role for antibody is in the opsonic process. (Reported fungicidal activity of serum more likely represents colony count reduction by the mechanical process of clumping with no real damage to the fungi.) Neutrophils are only moderately efficient in the killing of *C albicans*, and fungi can escape phagocytes by the process of germination. Pseudomycelia and mycelia can be destroyed by the mutual action of phagocytes that line up along their surface and release toxic products into extracellular vacuoles. Destruction of *C albicans* by neutrophils is oxygen-dependent, whereas nonoxidative mechanisms suffice for most other *Candida* species. Monocyte destruction of all *Candida* species is oxygen-dependent. Chymotrypsinlike cationic protein (CLCP), found in primary granules of human neutrophils, has antifungal activity. Cationic peptides present in alveolar macrophages also have been shown to have potent anti-*Candida* activity.

Cell-mediated immunity may be less crucial than adequate neutrophil function in disseminated candidiasis, whereas the converse appears to be true for chronic mucocutaneous candidiasis. Thus, adequate neutrophil function may protect the congenitally athymic nude mouse and the patient with AIDS from hematogenously disseminated *C albicans* infection.

Clinical Features

Once in the bloodstream, fungi disseminate to certain preferred target tissues, including the eyes, kidneys, meninges, skin, and myocardium. The renal tubule is an immunologically protected focus for *C albicans* replication. By budding into the renal tubules, fungi are able to transiently escape phagocytosis by PMNs, permitting them a temporal advantage in the course of infection. Mycelia enter the interstitial tissues and reseed the blood in a self-perpetuating mechanism. The susceptibility of a patient and the speed with which *Candida* disseminates are dramatically increased by the presence of leukopenia, especially in patients with myelotoxic cancer chemotherapy.

Immunologic Diagnosis

The diagnosis of disseminated candidiasis is made before death in fewer than 40% of cases. Although positive cultures are helpful, blood cultures are positive in fewer than 50% of autopsy-proved cases of dis-

semination. Tests for antibody (agglutinins, precipitins) are falsely negative in 30–70% of leukemic patients, perhaps because desperately ill patients may not form adequate antibody. When they do, it may be complexed to candidal antigens and thus not be detectable as free antibody. Tests for *Candida* antigens are more promising. Alpha-D-mannose, the principal antigenic polysaccharide component of the *Candida* cell wall, can be detected by EIA, RIA, or hemagglutination inhibition techniques; serum mannose and arabinitol can be detected by gas-liquid chromatography. Arabinitol accumulates in renal failure; hence, its concentration must be expressed as a ratio with creatinine. Among these tests, that for mannan appears to be the most promising. However, there will be uncertainty until a prospective trial is devised. It may be difficult to differentiate heavy *Candida* colonization from hematogenous dissemination, since both may be expected to liberate *Candida* antigens into the bloodstream.

Prevention

There is no available *Candida* vaccine.

Treatment

Systemic candidiasis is usually treated with amphotericin B, occasionally in combination with flucytosine. Mucosal disease responds well to ketoconazole, 200–400 mg orally daily.

4. SALMONELLOSIS

Salmonellae are gram-negative, aerobic, nonspore-forming rods that are found in the gastrointestinal tracts of humans and animals. The microorganisms may survive for variable periods of time in the environment. There are 3 principal *Salmonella* species: *Salmonella enteritidis* (humans and animals), *Salmonella choleraesuis* (swine), and *Salmonella typhi* (humans). More than 1800 serotypes of *S enteritidis* have been described. *S choleraesuis* and *S typhi* have only one serotype each.

Three principal syndromes result from *Salmonella* infection in humans: (1) gastroenteritis, the most common form of salmonellosis; (2) enteric fever; and (3) extraintestinal focal infections such as osteomyelitis, infected aortic aneurysms, etc.

A primary step in *Salmonella* pathogenesis is penetration of the intestinal mucosa by a mechanism that apparently includes digestion of the mucosal glycocalix. *Salmonella* species that produce the enteritis syndrome are found in endocytic vacuoles within mucosal cells. Salmonellae causing the enteric fever syndrome or extraintestinal invasive infection seem to be transported through mucosal cells in these vacuoles, next entering the lamina propria via the basal cell membrane. Subsequently, they reach the bloodstream and reticuloendothelial system.

In the gastroenteritis syndrome, salmonellae invade and destroy the bowel mucosa. PMNs appear to play at least some role in the host response. PMNs not only can kill many *Salmonella* species in vitro but are also prominent in the intestinal lesions and stools of patients with gastroenteritis. The lymphoid follicles of Peyer's patches provide a formidable first line of defense. After oral infection of mice, low numbers of salmonellae appear in Peyer's patches, multiply in 3 days to 10^5 organisms, but then decline to scarcity by 10 days after challenge. This does not appear to represent a passthrough to other target organs. Local immunity may be a consequence of chronic stimulation by gram-negative enteric bacilli and thus nonspecific. However, recent studies suggest that gut-associated lymphoid tissues (GALT) are capable of directly killing enteric pathogens by antibody-dependent cellular toxicity involving SIgA. *Salmonella* gastroenteritis is generally self-limited.

In enteric fever (usually due to *S typhi*), the host-parasite relationship is dramatically different. After ingestion, the microorganisms multiply asymptomatically in the gastrointestinal tract and result in a transient bacteremia. However, salmonellae appear susceptible to the complement-mediated bactericidal activity of normal human serum. This occurs through initial C1 binding, probably to the sugar portion of the core of lipopolysaccharides and is independent of antibody. Perhaps the salmonellae are protected within phagocytes and carried to the fixed macrophages of the reticuloendothelial system. The subsequent intracellular multiplication of *S typhi*, with production of a secondary sustained bacteremia, constitutes an essential pathophysiologic feature of enteric fever. Invasion of the biliary system results in the reentry of microorganisms into the gastrointestinal tract in massive numbers. Involvement of lymphoid tissue in the intestinal tract, principally Peyer's patches in the terminal ileum, leads to necrosis and ulceration. Typical clinical manifestations of typhoid fever include fever, headache, apathy, cough, prostration, splenomegaly, skin rash ("rose spots"), and leukocytopenia. The course of the disease is prolonged; relapses are common.

Factors that suggest the involvement of cell-mediated immune mechanisms in *Salmonella* enteric fever include the following:

(1) The occurrence of intracellular parasitism.
(2) The demonstrated ability to transfer immunity to normal animals adoptively with lymphocytes of immune animals.
(3) The absence of significant participation by PMNs in the infective process.
(4) When killed salmonellae or their extracts are administered to animals in Freund's complete adjuvant, solid immunity is established which is comparable to the immunity achieved by infection with attenuated microorganisms. This is a pattern characteristic of cell-mediated immunity. Conversely, administration of killed vaccines alone will generate high-titer antibodies and delay (but not prevent) infection. Passive immunization with immune serum is of no benefit.

(5) Delayed hypersensitivity skin tests with protein extracts of salmonellae convert to positive after animals are immunized with salmonellae in Freund's complete adjuvant.

The macrophage is important in host defense as well. Peritoneal exudate cells from immunized mice are far more active against *S enteritidis* than those from nonimmunized mice. This bactericidal activity depends in part on heat-stable opsonizing antibody. It is highly specific in that nonspecifically activated macrophages (*Listeria* infection) are no more effective than normal macrophages in reducing *S enteritidis* growth.

Immunologic Diagnosis

An increase in titer of agglutinins against the somatic (O) and flagellar (H) antigens of *S typhi* (Widal test) usually occurs during the course of typhoid fever, reaching a peak during the third week of illness. A 4-fold titer increase is held to be significant. Unfortunately, the test is far less specific than is generally appreciated, and titers may be elevated in many hyperglobulinemic states (chronic liver disease is one example). Furthermore, progressive increases in titer occur in association with many diseases not due to *Salmonella*. The use of the agglutination reaction as a diagnostic test should always be subordinated to direct cultural demonstration of the infecting microorganism. There are no commercial tests for cell-mediated immune responses to *Salmonella* extracts.

Prevention

Commercial typhoid vaccines consist of acetone-killed or heat-phenol-killed *S typhi;* they appear capable only of raising the minimum infecting dose of *S typhi* in vaccinated subjects. The Ty 21a strain of *S typhi* lacks the enzyme uridine diphosphogalactose-4-epimerase and is avirulent. When administered by the oral route, it has shown protective capability against subsequent typhoid fever. Under these circumstances, it would appear that a live attenuated vaccine should offer the best chance of protection.

Treatment

Chloramphenicol and ampicillin are the major agents used to treat enteric fever or bacteremic salmonellosis. Prolonged therapy is required, since microorganisms are sequestered in macrophages and presumably protected from the entry of antibiotics.

5. LISTERIOSIS

Listeria monocytogenes is a small, pleomorphic gram-positive rod that may be confused with nonpathogenic "diphtheroids" or with beta-hemolytic streptococci. It produces sporadic infections in humans, usually in the form of bacteremia and meningitis. Two features have elevated listeriosis to a position of prominence in the fields of infectious disease and immunology. First, it is clear that patients with depressed cell-mediated immunity resulting from lymphoreticular malignancy or the use of immunosuppressive drugs have an increased susceptibility to listeriosis. Second, infection with *L monocytogenes* has become an important model for studies of cell-mediated immune mechanisms in experimental animals An important observation made with *L monocytogenes*—since confirmed with other intracellular pathogens—is that immunity can be adoptively transferred to noninfected animals by means of lymphocytes from animals that have recovered from listeriosis.

In mice infected with *Listeria*, initial reduction in tissue counts is due to sequestration and killing by macrophages. PMNs play a prominent role somewhat later in the course of infection.

In humans, the infection appears to be self-limited and usually does not produce prolonged granulomatous disease. Indeed, listeriosis often presents as an acute purulent meningitis with a predominance of PMNs and a low cerebrospinal fluid glucose concentration. Nevertheless, there appears to be no question that cell-mediated immune mechanisms are critically important in control of this infection. The precise factors that dictate the virulence of this pathogen are unknown. There has been some suggestion that the complement system which readily opsonizes *Listeria* may actually "protect" the bacilli by facilitating their transport to a "protected" environment within monocytes.

INTRACELLULAR INFECTIONS IN WHICH LYMPHOCYTES & MACROPHAGES ARE DECISIVE IN RECOVERY & HUMORAL IMMUNE MECHANISMS PLAY NO PROTECTIVE ROLE

Major Immunologic Features

- Facultative or obligate intramacrophagic parasitism.
- Lymphocyte-macrophage interaction regulates immunity but may simultaneously mediate destruction of host tissues (bystander cell injury).
- Granulomas and giant cells characteristic of immune response.
- PMNs and opsonins inconsequential in protection.

General Considerations

Infections due to *Mycobacterium tuberculosis* and *Mycobacterium leprae* fulfill the above criteria particularly well. Although there may be considerable antibody synthesis in many mycobacterial infections (to the point of marked hypergammaglobulinemia in lepromatous leprosy), the antibody is broadly cross-reactive. Hence, its measurement is not of current practical significance. Mycobacteria are easily phagocytized in the absence of specific opsonins, perhaps because of an affinity between their lipid-rich cell walls and the lipids of macrophage cell membranes.

The second group of diseases in this section—histoplasmosis, coccidioidomycosis, brucellosis, and tularemia—are distinguished from diseases due to mycobacteria in that measurement of antibody is of definite practical value in diagnosis, prognosis, or both.

Diseases In Which Measurement of Antibodies Is Not Useful In Diagnosis or Prognosis

1. TUBERCULOSIS

M tuberculosis is a facultatively intracellular, aerobic, acid-fast bacillus naturally pathogenic only for humans. It produces no known toxins, and its virulence relates to its ability to survive and proliferate in mononuclear phagocytes. Recent studies suggest that *M tuberculosis* may evade the bactericidal activity of macrophages by preventing the fusion of enzyme-containing lysosomes with phagosomes containing the bacilli. Dead tubercle bacilli do not demonstrate this phenomenon.

Several microbial constituents appear to influence the pattern of host response: (1) Cord factor (trehalose-6,6′-dimycolic acid), the material responsible for the in vitro serpentine cordlike growth of *M tuberculosis*, inhibits migration of leukocytes and stimulates granuloma formation. (2) High-molecular-weight lipids and waxes are probably responsible for much of the tissue reaction to this microorganism. Up to 60% of the dry weight of the cell wall of *M tuberculosis* is composed of lipid—which may account for the relative impermeability to stains, acid-fastness, unusual resistance to killing by acid and alkali, resistance to the bactericidal activity of complement, and resistance to intracellular macrophage digestion of this microorganism. (3) Wax D and tuberculoproteins may be largely responsible for the production of tuberculin hypersensitivity and skin test positivity. Wax D has been detected for up to 56 days in experimentally induced skin lesions and may account for the chronicity of tuberculous lesions.

The clinical manifestations of tuberculosis are clearly a function of the immune status of the host. The immune status appears to be at least in part genetically controlled. Recent studies have strongly suggested an increased susceptibility to tuberculosis infection and subsequent dissemination in persons with HLA-Bw15 antigen. The development of a sensitized T lymphocyte population is a double-edged sword. On the one hand, macrophages are stimulated (activated) to enhanced antimicrobial activity with limitation of mycobacterial growth. On the other hand, normal (bystander) tissues are seriously damaged by the violence of the immune (hypersensitivity) response to the infective agent. Tissue damage may reflect the discharge of hydrolytic enzymes from dying macrophages as well as direct inflammatory effects of lymphocyte mediators such as lymphotoxin, MIF, and others.

During the early course of tuberculosis in experimental animals, there is suppression of delayed hypersensitivity and other indices of cell-mediated immunity. This has been associated with suppressor T lymphocytes and macrophages. More recently, arabinogalactan in the sera of tuberculous patients has been shown to suppress lymphocyte responses of normal subjects to PPD and phytohemagglutinin. These phenomena may account for the anergy seen in some patients with tuberculosis.

- Some investigators believe that immunity and tuberculin hypersensitivity are not inextricably linked. For example, macrophages may act to control *M tuberculosis* through nonphagocytic mechanisms by the secretion of antimycobacterial fatty acids. Furthermore, animals can be rendered tuberculin-sensitive by injecting them with wax D and tuberculoprotein; such animals are still entirely susceptible to infection with *M tuberculosis*. Conversely, certain RNA-protein complexes isolated from mycobacterial cells will induce high degrees of increased resistance to tuberculous infection without inducing tuberculin hypersensitivity. Finally, animals immunized against tuberculosis with a mycobacterial RNA-protein complex do not show increased resistance to infection with other intracellular parasites. These data suggest that the general phenomenon of macrophage activation with enhanced nonspecific killing of any intracellular microorganism may not be as important as specific, lymphocyte-mediated immunity, however that specificity is mediated.

Clinical Features

Tuberculosis is predominantly a pulmonary disease transmitted by aerosol. Hematogenous dissemination may allow secondary infection to develop in virtually any organ system. Major sections of standard textbooks are devoted to the clinical features of tuberculosis. This discussion will be limited to the immunologic perspective.

Pulmonary tuberculosis in the nonimmune patient is characterized initially by relatively unrestricted bacillary multiplication, with the development of infiltrative disease of the lower lobes or lower segments of the upper lobes. The inflammatory response is "exudative," being composed mainly of PMNs and monocytes. Tissue destruction and cavitation are not seen, and there may be remarkably few symptoms of infection, particularly in children. Bacilli spread via the lymphatics to regional lymph nodes and often reach the bloodstream. Infection is well tolerated for several weeks, during which time active immunity develops with resultant enhanced macrophage antimicrobial activity, curtailment of bacillary multiplication, resolution of the pneumonic process, and healing of extrapulmonary lesions. Simultaneously, a positive tuberculin skin test develops, reflecting the presence of specifically sensitized T lymphocytes. A hallmark of healed primary pulmonary tuberculosis is the **Ghon complex**, which generally consists of a calcified node in the lung parenchyma and enlarged, often calcified hilar nodes. It is crucial to realize that virulent tubercle

bacilli may persist for years, or even a lifetime, in such "healed" lesions. These organisms are different from replicating *M tuberculosis* in that they have unique antigens not shared with dividing *M tuberculosis*. In some cases, primary tuberculosis does not heal but goes on to produce cavitation (especially in adults). Patients may also develop fulminating disseminated (miliary) tuberculosis, typically associated with meningitis.

The pattern of infection in the immune (tuberculin skin test-positive) patient results in a totally distinct disease process in which there is extensive tissue destruction. **Postprimary, or reinfection, tuberculosis** may occur as a result of exogenous reinfection but more often reflects a recrudescence of old infection **(endogenous reinfection)** in response to intercurrent debilitating illness, advanced age, or immunosuppression. Lesions are located typically in apical or subapical pulmonary segments. Regional lymph node involvement is not conspicuous; disease is far more localized. Lesions are more likely to excavate and spread by bronchogenic dissemination within the lung rather than by lymphohematogenous spread beyond the lung. The inflammatory response is characterized by granuloma formation with giant cells and **caseation necrosis** (in which the necrotic tissue remains semisolid, with the consistency of cheese). Tissue destruction is profound; pulmonary fibrosis is common, and calcification may occur.

Reinfection tuberculosis may occur at any of the sites of original hematogenous dissemination resulting from primary tuberculosis. Renal, meningeal, genitourinary, and skeletal tuberculosis are beyond the scope of this discussion.

Immunologic Diagnosis

A. Tuberculin Skin Test: (See Chapter 18.) The tuberculin skin test provides no diagnostic information relative to a given acute illness unless it can be established that the skin test has converted from negative to positive in temporal relation to that illness. Otherwise, a positive skin test indicates only that the patient has experienced tuberculosis or a closely related mycobacterial infection at some time in the past. A positive tuberculin skin test indicates the presence of specifically sensitized T lymphocytes. A strongly positive skin test is thought to represent greater liability to active infection, since a larger mass of latent tuberculous disease would theoretically have to be present to maintain strong tuberculin hypersensitivity.

The principal tuberculin skin test preparation is **purified protein derivative (PPD).** PPD is prepared by ammonium sulfate precipitation of culture filtrate and is standardized in terms of biologic reactivity as "tuberculin units" (TU). One TU is the activity contained in a specific weight of Seibert's PPD lot No. 49608 in a specified buffer (PPD-S). First strength tuberculin has 1 TU, intermediate strength 5 TU, and second strength 250 TU of activity.

Of several ways to administer intradermal PPD, only the Mantoux procedure is reliable and reproducible. First-strength tuberculin is no longer used. Second-strength tuberculin is frequently cross-reactive with mycobacteria other than *M tuberculosis* (atypical mycobacteria) and thus lacks specificity. Intermediate-strength tuberculin is the material used for most skin-testing procedures. A positive response consists of 10 mm or more of erythema and induration appearing between 48 and 72 hours after the skin test antigen is applied. A negative skin test signifies either no tuberculosis infection or else the presence of anergy due to overwhelming infection or associated immunosuppressive illness (sarcoidosis, Hodgkin's disease). (See Chapter 18.) Anergy may be specific to PPD or broad to all antigens. It may be associated with depressed in vitro tests for cell-mediated immunity, such as lymphocyte blastogenesis. Anergy is frequently associated with increased numbers of T suppressor lymphocytes. Tuberculin sensitivity develops 2–10 weeks after infection. Patients with persistent anergy frequently convert their skin tests and in vitro tests to positive after a few weeks of treatment. Unfortunately, such "conversions" can be confused with "boosting" reactions in healthy people. Boosting occurs when a person with low-grade PPD reactivity is retested a week later and responds with a significantly larger reaction in the second test. This is due to the stimulus of the PPD antigen of the first test. The original infection may have been due to *M tuberculosis* occurring many years before or to nontuberculous mycobacteria that cross-react with PPD. To avoid mistaking boosting for real conversion, it is recommended that persons participating in regular skin testing programs who, within a period of 2 years, exhibit an increase in diameter of induration of at least 6 mm, with a resultant change in the diameter of skin test reactivity from < 10 mm to > 10 mm of induration, be considered newly infected.

B. Other Tests: There has been continuing interest in using in vitro tests to diagnose tuberculosis. The rationale is that these tests may be positive at a time when skin tests are negative. A recent study has cast doubt on the use of lymphocyte transformation assay. In 58 children with active tuberculosis, suspected tuberculosis, or no tuberculosis, there was complete correlation of the skin test reaction with the lymphocyte transformation reaction. Thus, the lymphocyte transformation test represented no improvement over the simpler skin test. There has also been interest in the use of antibody assays to diagnose tuberculosis. ELISA assays for antibody to PPD and to *M tuberculosis* antigen 6 (a major cytoplasmic protein antigen) have shown promise in the serologic diagnosis of pulmonary and bone-joint tuberculosis, respectively. Active work is also under way to develop specific tests for tuberculosis employing monoclonal antibody and recombinant DNA techniques. Finally, recent studies suggest that tuberculostearic acid, a fatty acid characteristic of microorganisms in the order Actinomycetales, may be identifiable in the sputum of patients with pulmonary tuberculosis by gas chromatography/mass spectrometry with selected ion monitoring. Un-

der these circumstances, it may soon be possible to diagnose tuberculosis more rapidly than by current bacteriologic techniques. Hemagglutinating antibody, radioimmunoassay, and other techniques have been used. The issue of testing is controversial, and there is still no convincing evidence that techniques other than the skin test will aid in diagnosis.

Differential Diagnosis

Infiltrative and cavitary pulmonary tuberculosis must be considered in the differential diagnosis of a plethora of pulmonary diseases. Weight loss, night sweats, and hemoptysis are common clues to reinfection tuberculosis. Discussion should be sought in standard medical textbooks.

Prevention

Living avirulent tubercle bacilli—especially BCG, an attenuated strain of *Mycobacterium bovis*—have been used to stimulate resistance to infection in persons at greater than normal risk of exposure to tuberculosis. Such immunization may provide many years of protection from tuberculosis infection, the principal disadvantage being conversion of the tuberculin skin test and hence unavailability of the test as a clue to exposure to *M tuberculosis*. BCG vaccine has never achieved popularity in the USA.

An alternative method of providing protection against tuberculosis is to treat all patients with recent skin test conversions with isoniazid for 1 year. The numbers of infecting microorganisms are low; thus, multiple drug regimens are not required. Recent data indicating the hepatotoxicity of isoniazid have led to greater care in selection of patients for isoniazid prophylaxis so that only those who definitely require the drug will be treated in this way. It is no longer suggested that anyone with a positive skin test, regardless of age or of the duration of the reaction, receive isoniazid as a matter of course.

Treatment

Cavitary pulmonary tuberculosis and disseminated forms of tuberculosis require treatment with combinations of drugs (isoniazid plus rifampin or other combinations) to retard the ascendency of naturally occurring drug-resistant mutants found in small numbers in any large population of *M tuberculosis*. Other texts should be consulted for more detailed discussions of the complicated problem of the treatment of tuberculosis.

Complications & Prognosis

Some of the complications of tuberculosis have already been mentioned. Meningitis, renal infection, etc, are discussed in other texts. Rarely—but especially in Scandinavian countries—tuberculosis may be complicated by the occurrence of erythema nodosum. This presumably reflects the presence of circulating immune complexes. Amyloidosis is an immunologic complication that may occur in patients with long-standing infection.

2. LEPROSY

M leprae is an obligate intracellular acid-fast bacillus which has a unique ability to invade nerves and a preference for growth in cool areas of the body. *M leprae* has never been cultivated successfully in vitro but will multiply to a limited extent in the footpads of mice. More widespread infection occurs in animals thymectomized at birth, emphasizing the crucial role of thymus-mediated immunity in this infection. Armadillos are susceptible to disseminated infection without the necessity for prior immunosuppression.

M leprae produces a remarkably broad spectrum of clinical disease ranging from **tuberculoid leprosy** at one extreme to **lepromatous leprosy** at the other. The pattern of infection is intimately related to the underlying degree of cell-mediated immunity. Like *M tuberculosis*, the leprosy bacillus elaborates no destructive enzymes or toxins; disease production is related directly to the ability to survive macrophage residence. Recent electron microscopic studies suggest that *M leprae* may evade macrophage antimicrobial activity by escaping from phagolysosomes to lie free in macrophage cytoplasm. The significance of these observations remains to be established.

Lepromatous leprosy is characterized by the virtual absence of an inflammatory response to *M leprae*. Thus, bacillary infiltration of tissues is extensive, and tissue destruction is minimal until very late in the disease. Lepromatous lesions consist of macrophages heavily parasitized with *M leprae*. The T cells in the lesions are devoid of CD4 helper cells and consist almost exclusively of CD8 supressor cell populations. This pattern of lymphocyte distribution is also present in the peripheral blood. In tuberculoid leprosy, the converse is true: the immune response is severe enough to damage or destroy bacilli and the nerves they infect at the outset. The tuberculoid infiltrates contain well-organized epithelioid and giant cell granulomas, only remnants of bacilli, and a predominant helper cell subset. Borderline leprosy refers to the broad sweep of intervening disease between the lepromatous and tuberculoid "poles" and accounts for most clinical infections. Borderline leprosy is characterized by clinical and immunologic instability.

M leprae is traditionally regarded as a feeble pathogen, requiring intimate and prolonged contact for transmission. However, there are so many exceptions that leprosy has also been considered an easily transmissible infection in populations where only a few persons are sufficiently susceptible to permit development of clinically apparent disease. Unfortunately, there is no diagnostic skin test to assist in identifying persons who have experienced asymptomatic infection. Recent studies based upon examination of *M leprae*-specific lymphocyte activation in asymptomatic leprosy contacts suggest that acquisition of infection (sensitization) is relatively common despite the rarity of symptomatic disease. (Whether the blastogenesis test is specific for *M leprae* is unclear.)

The factors that dictate susceptibility to clinical in-

fection remain uncertain. However, it is clear that inability to respond to the lepromin skin test (see below) is characteristic of patients with lepromatous leprosy and that lepromin anergy often persists despite apparent cure of disease. Thus, in a patient susceptible to lepromatous leprosy, an immune defect related to impaired recognition of *M leprae* is present. Whether the defect is predominantly one of macrophages or of lymphocytes remains contested. Genetic studies involving HLA typing indicate an HLA-linked recessive gene leading to the tuberculoid form of leprosy.

Clinical Features

A. Lepromatous Leprosy: Lepromatous leprosy is manifested by widespread bacillary invasion of the integument (except in warm skin folds) and the cooler mucous membranes (especially the nose, which may be a source of infective aerosols). Diffuse bacillary invasion of the facial tissues results in the characteristic leonine facies of advanced disease. There is continuous heavy bacteremia (10^5–10^6 bacilli/mL), which is well tolerated; liver, spleen, and bone marrow are loaded with microorganisms. Although peripheral nerves are heavily invaded by *M leprae*, nerve destruction does not occur until late in the illness, reflecting the poor immunologic response to infection. Histologic findings reflect the lack of immune response.

B. Tuberculoid Leprosy: This form of the disease is characterized by the presence of no more than one or 2 extremely well demarcated skin lesions with anesthetic atrophic centers and erythematous, raised edges. A palpable nerve trunk can often be found in the vicinity of the skin lesion. Histologic findings resemble those of sarcoidosis (epithelioid cell foci surrounded by well-defined zones of lymphocytes); however, the cutaneous nerves are destroyed by the tuberculoid leprosy granuloma, whereas nerves are spared in sarcoidosis. Rare acid-fast bacilli may be found in nerve remnants by careful serial sectioning of skin lesions. Caseation necrosis occurs very rarely in leprosy but may be seen in the nerves of patients with tuberculoid leprosy when there is a particularly vigorous immune response.

C. Borderline Leprosy: So-called borderline leprosy accounts for a broad spectrum of clinical disease. The more nearly lepromatous a case of borderline leprosy is, the more plentiful the bacilli, the more numerous the skin lesions, the less well defined their edges, and the less pronounced the anesthesia. The more nearly tuberculoid a case of borderline leprosy is, the fewer the number of lesions (and bacilli), the sharper their edges, the more pronounced the degree of anesthesia, the greater the chances for finding enlarged peripheral nerves, and the more lymphocytes on histologic examination.

Immunologic Diagnosis

A. Lepromin Skin Test: Lepromin has no diagnostic usefulness; lepromin skin test reactivity is common in normal persons by virtue of sensitivity to cross-reacting mycobacterial antigens and foreign skin proteins. Lepromin is prepared from a homogenate of lepromatous skin nodules; the extracted leprosy bacilli are variably purified. In a person who is *known* to have leprosy, lepromin reactivity is diagnostic of tuberculoid or near-tuberculoid disease, whereas lepromin anergy carries the prognosis for progression to lepromatous leprosy in the absence of treatment. Treated patients who fail to recover lepromin reactivity are at risk of relapse and should receive antileprosy chemotherapy for life.

B. Serologic Tests: Recent studies indicate that *M leprae* possesses 3 specific phenolic glycolipids, one of which (GL-I) is unique to that microorganism. The terminal 3,6-di-O-methyl glucose of GL-I is a sugar that has not been found previously in nature. High levels of anti-GL-I IgM antibodies are found in the sera of 96% of untreated lepromatous leprosy patients but not in the sera of patients with tuberculoid leprosy or atypical mycobacterial infections. The 3,6-di-O-methyl-β-D-glucopyranosyl substituent has been recognized as the primary antigenic determinant in GL-I, and a glycoprotein conjugate has been synthesized that offers the possibility of a specific hapten diagnostic reagent. Monoclonal antibodies generated to GL-I also show diagnostic promise. The phenolic glycolipid antigens are located on the outer surface of *M leprae*, are present in profusion, and undoubtedly influence host response to the bacillus.

Differential Diagnosis

Leprosy should be suspected whenever an anesthetic skin lesion is found. Skin biopsies with stains for mycobacteria should be used to seek histologic proof of diagnosis since even apparently full-blown lepromatous leprosy may be confused with post-kala azar dermal leishmaniasis. (The striking immunologic similarities between leishmaniasis [see Chapter 35] and leprosy are noteworthy as well.) The essential histologic criterion for a diagnosis of leprosy is the presence of acid-fast bacilli in nerves. More specialized sources should be sought for details of the diagnosis of leprosy.

Prevention

About 10% of untreated household contacts of lepromatous patients eventually develop leprosy; the risk is higher for children than adults. Prophylactic dapsone therapy may be of value in blocking disease transmission within families. The efficacy of BCG vaccine in the prevention of leprosy has not been established. However, mixtures of heat-killed *M leprae* and viable BCG appear to favorably influence the course of active leprosy infection. It has been postulated that the BCG stimulates cell-mediated immunity to antigenic components of *M leprae* toward which the host had not previously reacted. Although the mechanism by which this combination elicits effects is speculative, there is interest in its use for prophylaxis in lepromin skin test-negative contacts of leprosy patients.

Treatment

The mainstay of leprosy treatment is dapsone (diaminodiphenylsulfone, DDS). Rifampin is bactericidal for *M leprae* and promises to be very useful in treatment; its principal problem is one of expense. Clofazimine, a phenazine dye, has direct anti-*M leprae* activity but also appears capable of stimulating macrophage bactericidal activity (at least for *L monocytogenes*). In high doses, clofazimine also suppresses erythema nodosum leprosum activity (see below).

Immunologic Complications

There are 5 principal immunologic complications of leprosy:

A. "Antigen Overload": In patients with lepromatous leprosy, lepromin anergy permits the accumulation of astounding numbers of *M leprae* (10^9/g of skin, 10^5–10^6/mL of blood, 10^7/mL of nasal secretions, 10^{12} total body). Associated with the massive accumulation of foreign antigen are a variety of immunologic aberrations. Humoral abnormalities include polyclonal hypergammaglobulinemia, cryoglobulinemia, rheumatoid factor, biologic false-positive syphilis serology, and hyperglobulinemia-related latent distal renal tubular acidosis. Cellular abnormalities include depletion of paracortical (T) lymphocytes from lymph nodes, decreased numbers of circulating T cells, anergy to skin tests with recall antigens, impaired skin graft rejection, and impaired lymphocyte activation. All abnormalities are reversible with treatment, with the general exception of lepromin anergy.

The relationships that exist among antibody production, delayed hypersensitivity, and cell-mediated immunity in patients with leprosy are shown in Fig 30–3. In localized (tuberculoid) disease, serum antibody titers are low and delayed hypersensitivity and cell-mediated immunity maximal. In disseminated (lepromatous) disease, the opposite relationships obtain.

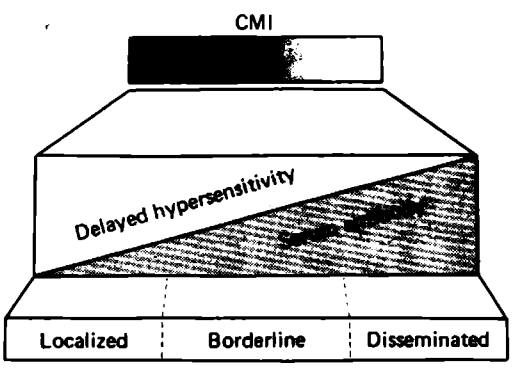

Figure 30–3. A schematic representation of the relationships among antibody production, delayed hypersensitivity, and cell-mediated immunity (CMI) as related to severity of intracellular infection. (Reproduced, with permission, from Bullock WE: Anergy and infection. *Adv Intern Med* 1976; **21**:158.)

This precise relationship can be applied without significant modification to a broad variety of other intracellular infections including coccidioidomycosis, histoplasmosis, leishmaniasis, and tuberculosis. The factors underlying depression of cell-mediated immunity and delayed hypersensitivity in disseminated infection are unclear but may relate to interruption of normal lymphocyte (predominantly T cell) traffic by granulomatous involvement of the periarteriolar lymphocytic sheaths of splenic white pulp and paracortical areas of lymph nodes. In addition, serum or plasma factors may be present that impair the response of lymphocytes to microbial antigens. Finally, there is evidence that intense stimulation of suppressor T cell function in certain intracellular disease processes may result in apparent deficiency of cell-mediated immune mechanisms.

B. Erythema Nodosum Leprosum: Erythema nodosum leprosum is a complication of lepromatous leprosy and results from an Arthus-type immune reaction in the skin and from the deposition of circulating immune complexes in the joints and kidneys. Nearly 50% of lepromatous patients will acquire erythema nodosum leprosum; it may occur spontaneously but more often follows initiation of chemotherapy. Erythema nodosum leprosum is characterized histologically by vasculitis and panniculitis and clinically by recurrent crops of up to hundreds of red, hot, tender skin lesions occurring all over the body. This complication is associated with severe systemic symptoms. The recent discovery that erythema nodosum leprosum is rapidly eradicated by thalidomide has revolutionized the therapeutic approach to this difficult problem.

C. Lucio Phenomenon (Erythema Necroticans): The Lucio phenomenon is a complication of certain forms of lepromatous leprosy. Necrotizing vasculitis produces crops of large polygonal lesions characterized by ulceration and sloughing of large areas of skin. It may be peculiar to specific ethnic groups.

D. Reversal and Downgrading Reactions: These "reactions" are characteristic of borderline forms of leprosy and represent abrupt shifts in cell-mediated immunity, often with striking nerve damage. Preexisting quiescent skin lesions become abruptly red, hot, and tender. Granulomas may break up and lymphocytes become sparse (downgrading reaction), or skin lesions may show evidence of acquisition of immunologic activity with influx of lymphocytes (reversal reaction). These immunologic complications may be very severe, necessitating treatment with corticosteroids. Thalidomide is not effective.

E. Amyloidosis: Secondary amyloidosis is an important cause of renal failure and death in patients with advanced leprosy. Curiously, this complication appears to be far more common in Caucasians than other ethnic groups.

Diseases in Which Measurement of Antibody Is Useful in Diagnosis or Prognosis

The diseases in this group share many of the immunopathologic features of the mycobacterial infections, particularly tuberculosis; a major difference is that antibodies, though subserving no protective function, may assist in establishing a diagnosis or prognosis.

3. HISTOPLASMOSIS

Histoplasma capsulatum is a dimorphic fungus that exists in mycelial form in nature and as an intracellular yeast in humans and susceptible animals. Despite its name, this fungus is not encapsulated.

In the USA, the midwestern portion of the country, especially the Mississippi and Missouri River valleys, is the area of highest endemicity.

Exposure to *H capsulatum* in the endemic areas is usually frequent and often heavy. Avian droppings of chickens, starlings, and other birds provide a compost for luxuriant growth. When infected soil is disturbed, spores are aerosolized and inhaled. These are distributed throughout the lungs and presumably enter macrophages, where they convert to the parasitic yeast form. In the first few weeks after exposure, cell-mediated immune responses develop. The results are a positive histoplasmin skin test, lymphokine production, death or cicatrization of the intracellular yeast, and fibrocalcific healing at the site of primary infection. This last process is expressed by "buckshot" calcifications in the lung and spleen (fungemia is part of the initial process).

Cell-mediated immunity is critical to host defense. If there is failure of cell-mediated immunity, the yeasts proliferate unchecked in macrophages. This results in uncontrolled disseminated histoplasmosis with diffusely enlarged reticuloendothelial organs such as the liver, spleen, and lymph nodes. Athymic (nude) mice develop this form of disease. They can be protected by thymus transplantation or provision of T lymphocytes. Further, in murine histoplasmosis there is activation of splenic suppressor macrophage activity. This depresses cell-mediated immune responses. Activity of these cells is maximal in the first weeks after infection. Their role in host defense is not known. As with other fungi, transferrin may inhibit the growth of *H capsulatum* by sequestering iron from the fungus.

It is of note that the healing process may be more destructive of tissue than the disseminated disease. If extensive fibrosis occurs, the buckshot calcification may enlarge into a histoplasmoma, or mediastinal fibrosis may occur. There is much granuloma and inflammatory reaction with few fungi seen. In disseminated disease, granuloma formation is poor; macrophages are loaded with yeasts, but there is little necrosis.

Clinical Features

Several distinctive syndromes of histoplasmosis are recognized:

A. Primary Histoplasmosis: Primary histoplasmosis is generally asymptomatic or attributed to an episode of "flu"; it is recognized in retrospect only by the acquisition of histoplasmin skin test reactivity. In heavy exposures, patients are more likely to manifest cough, malaise, and fever. In most cases, the disease is self-limited, with calcified granulomas representing the residue.

B. Acute Disseminated Histoplasmosis: In the patient who fails to contain the infection at the outset, the result may be acute disseminated histoplasmosis. This complication occurs typically but not exclusively in children. It is characterized by fever, hepatosplenomegaly, anemia, and leukopenia. Untreated, it is highly lethal; death occurs in days to weeks.

C. Acute Reinfection Histoplasmosis: The patient with histoplasmin sensitivity who continues to reside in a histoplasmosis-endemic area is subject to reexposure to the fungus. Mild reexposures may be asymptomatic, serving only as "boosters" to maintenance of histoplasmin skin test reactivity. In patients possessing cell-mediated immunity to *H capsulatum*, the course of illness resulting from a subsequent massive reexposure is characterized by a severe pulmonary inflammatory response. Acute reinfection pulmonary histoplasmosis is thus related more closely to host hypersensitivity than to fungal invasiveness and is usually self-limited.

There is no event comparable to massive exogenous histoplasmosis reexposure in patients with tuberculosis, since neither the environment nor infected patient contacts harbor mycobacteria in the concentrations necessary to produce this intense immunologic reaction.

D. Chronic Pulmonary Histoplasmosis: Chronic pulmonary histoplasmosis bears a striking resemblance to pulmonary tuberculosis (with which it was historically confused and with which it may coexist). It occurs predominantly in middle-aged white cigarette smokers, especially those with chronic obstructive pulmonary disease and, undoubtedly, recurrent exposure to *H capsulatum*. At the outset, one or more areas of infiltration or cavitation usually develop in the peripheral apical-posterior segments of the lungs. The lesions may slowly heal, contracting into a scarred band; or they may cavitate, with progressive cavitary enlargement and attendant pulmonary destruction. New lesions may appear as old ones are resolving, which suggests that breakdown of old lesions may lead to antigenic spillover into new sites, with development of secondary inflammatory processes.

Chronic pulmonary histoplasmosis is not associated with extrapulmonary dissemination, as cellular immunity is basically intact. The disease appears to persist because of the presence of underlying abnormal pulmonary anatomy and perhaps aberrantly increased immune response.

E. Chronic Disseminated Histoplasmosis: Chronic disseminated histoplasmosis occurs in patients with defective cell-mediated immunity. This syndrome reflects generalized *Histoplasma* invasion of the reticuloendothelial system. Clinical findings thus include fever, anemia, leukopenia, hepatosplenomegaly, lymphadenopathy, and wasting. Childhood forms include widespread dissemination with signs of marrow suppression and splenic involvement. Adult forms are usually chronic, with little marrow dysfunction and more focal lesions, particularly in the oral mucosa. The most serious (and surprisingly common) complication of this form of histoplasmosis is adrenal gland invasion and destruction.

Immunologic Diagnosis

Despite the close correlation of immunologic and clinical status, immunologic tools are of little help in establishing a clinical diagnosis. There are several reasons for this: (1) The histoplasmin skin test is so frequently positive in endemic areas that a positive skin test is meaningless in an individual patient and merely reflects a prior infective encounter with the fungus. (2) The serologic titers are often boosted artifactually by a previous positive skin test. Improved skin test antigens are now being prepared from yeast phase fungi. These have less cross-reactivity and apparently do not influence the serologic assays. Other efforts have been made to circumvent problems of skin testing by measuring complement fixation titers against yeast phase antigens (since histoplasmin is prepared from mycelial phase microorganisms). This is done in the hope that the yeast phase titer will rise only in worsening disease, in contrast to the false mycelial phase elevation from skin testing. This works better in theory than in practice, since both titers often rise after skin testing. Even a coccidioidin skin test may produce an elevation of *Histoplasma* yeast phase complement-fixing antibody titers. (3) Other serologic assays, such as precipitins and agar gel diffusion, have been used in an attempt to identify the patient with active histoplasmosis. None of these are thoroughly reliable. (4) The histoplasmin complement fixation antibody is elevated in only about half of patients with disseminated histoplasmosis. Many are therefore missed by this test. (5) There is frequent serologic cross-reaction with antigens of *Blastomyces dermatitidis*. Because blastomycosis occurs in the histoplasmosis-endemic areas, this could lead to misdiagnosis. (6) Antigens of these 3 fungi cross-react extensively in tests of cell-mediated immunity such as lymphocyte blastogenesis.

One might ask whether there is any value at all in immunologic testing. The response is a qualified "yes" for serology and a very limited "yes" for the skin test. The serologic titer might conceivably be helpful in a very ill patient with clinical findings suggesting histoplasmosis, one in whom it may be inappropriate to withhold treatment for up to 4 weeks pending culture results. If strongly elevated, and if not complicated by a preceding skin test, the high titer may be grounds for a presumptive diagnosis of histoplasmosis pending the results of cultures.

Histoplasmin skin tests are most useful (1) in epidemiologic surveys, (2) in evaluation of a patient for skin test anergy as part of a general immunologic evaluation for a reason unrelated to histoplasmosis, and (3) in assessment of cell-mediated immunity in a mycologically confirmed histoplasmosis patient. This is rarely necessary and should be done for prognostic purposes, not for diagnosis. Immunohistochemical tissue staining may also be helpful in detecting *H capsulatum*.

Marked elevation of IgE occurs in active histoplasmosis. The cause of this phenomenon and its possible diagnostic value, if any, have not been determined.

In summary, many physicians use immunologic methods to diagnose histoplasmosis. Because of the pitfalls outlined above, many of these diagnoses are incorrect. The best and most absolute method is to culture *H capsulatum* from tissue of the infected patient.

Prevention

Animals can be immunized with *Histoplasma* extracts in Freund's complete adjuvant or by sublethal infections with virulent *H capsulatum*. However, there are no suitably attenuated strains for human use. Furthermore, it is conceivable that immunization could adversely affect patients in whom hypersensitivity may play the major role in disease production.

Treatment

Prolonged amphotericin B has been the mainstay of medical therapy. Ketoconazole appears effective and less toxic. Several patients with histoplasmosis, both pulmonary and disseminated, have been treated with transfer factor; results are inconclusive.

Complications & Prognosis

These may include bronchial obstruction by calcified *Histoplasma* granulomas that become broncholiths. Destruction of lung by chronic pulmonary histoplasmosis may lead to secondary bacterial infection and respiratory insufficiency as a late complication, and even treated patients should be followed chronically for this problem.

The prognosis for spontaneous recovery from acute pulmonary histoplasmosis is excellent, whereas patients with chronic cavitary pulmonary histoplasmosis are prone to relapse even after treatment with amphotericin B. Surgical resection is often beneficial. Indications for surgery are unclear. Disseminated forms of histoplasmosis are fatal in more than 80% of instances in the absence of treatment with amphotericin B.

4. COCCIDIOIDOMYCOSIS

Coccidioides immitis, the etiologic agent of coccidioidomycosis, is a biphasic fungus that exists as a mycelium (mold) in its natural environment and as a unique endosporulating spherule in the infected host.

Dissolution of alternating cells in the segmented mycelium frees the intervening arthroconidia that are easily dispersed by air currents. When inhaled, the arthroconidia (2 × 5 μm in their rectangular dimensions) undergo progressive enlargement to produce spherical cells (spherules) that may reach 30–80 μm in diameter. Segmentation of the spherule cytoplasm results in the production of hundreds of endospores that are released when each spherule ruptures. Each endospore then develops into a new spherule. This morphogenic cycle is unique to *C immitis;* it bears emphasis that there is no yeastlike stage.

Coccidioidomycosis is unique to the Western Hemisphere. In the Southwestern USA (especially Arizona, California, and Texas) and contiguous areas of Mexico, 50–100 thousand cases occur annually. It was first described in Argentina and occurs in other parts of South and Central America as well.

Because *C immitis* is a complex microorganism, the host must be prepared to deal with arthroconidia, followed by spherules and endospores. Each presents unique problems for host defenses. Arthroconidia possess an antiphagocytic surface (the hyphal outer wall layer) and, even when ingested by phagocytes present in the lung (eg, PMNs and alveolar macrophages), are killed inefficiently. Spherules reach extraordinary sizes (30–80 μm in diameter) and are difficult for individual phagocytes to ingest. The joint activity of PMNs and macrophages appears necessary for their destruction in vitro, but their vulnerability to these cells in vivo is uncertain. Spherules release endospores by the hundreds, taxing immunologic reserves. Young endospores, freshly released from spherules, are bound together by a fibrillar matrix that appears difficult for phagocytes to penetrate. By the time the matrix has dissipated, the endospores are already young spherules with thick cell walls. Generally speaking, PMNs appear to have little capacity to deal definitively with any of the forms of *C immitis*, even though these cells are commonly present within lesions. The role of eosinophils, also commonly present in infected tissues, is uncertain. It is generally considered that cell-mediated immunity (ie, macrophages and sensitized lymphocytes) constitutes the principal host defense mechanism in coccidioidomycosis. Although endospores are ingested by macrophages, they do not appear to be killed unless lymphokines from sensitized lymphocytes stimulate the fusion of phagosomes (that contain the organisms) and lysosomes (containing fungicidal and digestive enzymes). In vitro studies suggest that complement-fixing antibody is opsonic for *C immitis*. The immunity associated with coccidioidin (or spherulin) skin test positivity is solid. Reinfection is extraordinarily rare except under conditions of heavy arthroconidial reexposure (eg, in a laboratory accident).

Although rates of disease acquisition are related predominantly to environmental exposure, a number of host-determined factors influence the subsequent course of events.

Race. On the basis of the results of clinical and autopsy series, blacks are approximately 10–15 times as likely as whites to experience extrapulmonary coccidioidal dissemination. This appears to be true even under identical conditions of exposure (eg, in military trainees). The explanation is obscure; there is no regular association with HLA haplotypes. Purportedly increased "racial" susceptibility of Filipinos, Orientals, Native Americans, and Mexican-Americans is not based on firm epidemiologic data.

Sex. Under ordinary circumstances, men are more likely than women to experience coccidioidal dissemination. This may relate to their greater opportunity for high inoculum environmental exposures.

Pregnancy. If coccidioidomycosis is acquired during the second or third trimester of pregnancy, women become extremely prone to coccidioidal dissemination—often with fatal outcome. Increased susceptibility may be related partially to the modest immunosuppression that characterizes advanced pregnancy. However, recent studies indicate that *C immitis* may be directly stimulated by elevated levels of free estradiol and progesterone that occur in the sera of pregnant women.

Age. Carefully conducted studies in Native American populations living in the endemic zone of Arizona suggest that the very young and the very old are more susceptible to coccidioidal dissemination. In the case of the young, this may relate to greater opportunities for direct soil exposure afforded by outside play. The explanation for increased dissemination in the very old may be reduced immunocompetence or intercurrent debilitating disease.

Immunosuppression. Intact cell-mediated immunity appears to be essential for containment of *C immitis* infection. Patients with depressed cell-mediated immunity are more susceptible to coccidioidal dissemination.

Clinical Features

More than 60% of cases of coccidioidomycosis are acquired asymptomatically. Clinical disease may present as a pulmonary problem with pneumonia, abscesses, and thin-walled cysts or as a disseminated disease process. The most devastating form of the disease is represented by meningeal involvement, which is almost invariably complicated by obstructive hydrocephalus.

Immunologic Diagnosis

A. Skin Tests and In Vitro Correlates of Cell-Mediated Immunity: There are 2 preparations for detecting delayed hypersensitivity to *C immitis*. **Coccidioidin** is prepared from culture filtrates of the mycelial-arthroconidial phase and has been the standard preparation since the late 1930s. **Spherulin** represents the soluble fraction from lysed *C immitis* spherules and has been available for less than 5 years. Although spherulin has been claimed to identify up to one-third more skin test reactors than coccidioidin, these data are subject to question. Either preparation is acceptable for diagnostic purposes. Coccidioidin is or-

dinarily used in a dilution of 1:100; the equivalent concentration of spherulin is referred to as "usual skin test strength." The criterion for positivity with either test is a 5×5 mm area of induration and erythema.

In general, patients with primary pulmonary coccidioidomycosis develop sensitivity to coccidioidin within 3 days to 3 weeks after the onset of symptoms. If the exact time of arthroconidial exposure can be established (eg, laboratory accident), coccidioidin reactivity may become detectable as early as 10–12 days later.

Patients with an established diagnosis of coccidioidomycosis and a negative coccidioidin or spherulin skin test are considered to be manifesting a poor immune response to infection. Some of these patients may respond to 10-fold more concentrated preparations of coccidioidin or spherulin or to undiluted skin test material. In this way, the extent of anergy can be titrated.

In vitro parameters of cell-mediated immunity also correlate with disease activity but are less helpful. The MIF is negative in most patients with active disease and is therefore not a helpful index of severity. The lymphocyte transformation reaction is negative in the most severe cases; in milder cases, lymphocyte transformation often does not correlate well with other tests of immune response. In vitro test responses, like the skin test, may convert to positive with clinical improvement, though they do not always do so simultaneously.

Expressions of cell-mediated immunity are also related to serologic changes. Serum-blocking factors (probably immune complexes) have been described that can depress skin test or lymphocyte transformation reactivity. Their relationship to clinical disease, severity, or prognosis is uncertain.

B. Serologic Tests:

1. Precipitin test–Precipitating antibody is detected by mixing serum and coccidioidin in a test tube (tube precipitin test, TP), by latex particle agglutination (LPA), or, most satisfactorily, by immunodiffusion (IDTP). Because there may be 10% or more false-positive results with the LPA technique, this test should be regarded only as an indicator of need for further serologic evaluation. In contrast, the TP and IDTP tests appear to be specific for coccidioidomycosis. Precipitins are IgM class antibodies and occur early in the course of infection. When the TP method is used, 53% of sera are positive during the first week of illness; 91% during the second and third weeks; and 86% during the fourth week. Thereafter, rates of positivity diminish quickly. In patients who develop infection in the absence of any symptoms (asymptomatic skin test converters), the precipitin test may never become positive. In patients with mild disease, a positive precipitin test may not be followed by a positive complement fixation antibody response.

Tests for precipitins are reported either as "positive" or "negative"; quantification has not been found to be of value. Measurement of precipitins in the cerebrospinal fluid is without diagnostic value.

2. Complement fixation (CF) test–Complement fixation antibody can be measured either by classic hemolytic assays or, far more conveniently, by immunodiffusion methodology (IDCF). There is some variation in complement fixation titers from laboratory to laboratory, so that noncritical attempts at comparison can be misleading.

In asymptomatic skin test converters, the complement fixation antibody response may never develop. When complement fixation antibody does develop in patients with primary coccidioidomycosis, it characteristically follows the precipitin response (as IgG follows IgM). Complement fixation titers seldom exceed 1:16 in uncomplicated primary infections and usually decrease shortly after recovery. However, antibody may persist in the serum in low titers (1:2 to 1:8) for many years.

Properly performed, the complement fixation test can be a valuable guide to diagnosis (conversion from negative to positive, or documentation of a 4-fold titer increase during a current illness) and especially to prognosis. High or rapidly increasing complement fixation titers (especially in association with negative skin tests) are generally associated with extrapulmonary dissemination, whereas a significant decrease in the complement fixation titer during therapy (especially with maintenance or recovery of skin test positivity) is generally associated with a favorable prognosis.

The sequence of immunologic responses during primary coccidioidomycosis is indicated in Fig 30–4. A positive precipitin test coupled with a negative complement fixation test indicates early primary disease. As the precipitin antibody titer falls, the complement fixation titer rises, especially in the presence of long-active disease. Therefore, a positive complement

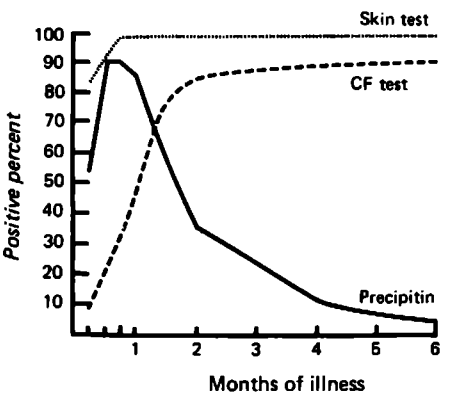

Figure 31–4. Relationships among immunologic reactions in symptomatic primary coccidioidomycosis, relating time of appearance and duration to the frequency of positive reactions. (Reproduced, with permission, from Huppert M: Serology of coccidioidomycosis. *Mycopathol Mycol Appl* 1970;41:108. Courtesy of W. Junk N.V., Publishers, The Hague.)

fixation test with or without precipitin reactivity indicates chronic disease.

The relationship between complement fixation titer and severity of illness is shown in Fig 30–5. The complement-fixing antibody titer correlates inversely with the competence of cell-mediated immunity. In one study, 80% of patients with a coccidioidin complement fixation titer of 1:32 or less had a positive coccidioidin skin test, compared to only 41% of those with higher serologic titers. Furthermore, DNCB sensitization, another index of cell-mediated immune function, was markedly depressed in those with high complement-fixation titers.

Serologic tests are particularly important in diagnosing coccidioidal meningitis. *C immitis* is difficult to recover from cerebrospinal fluid, and there may be no other identifiable foci of disease. Unfortunately, 10–25% of patients with coccidioidal meningitis have a negative complement fixation test in the cerebrospinal fluid. Diagnosis may rest upon finding lymphocytosis and hypoglycorrhachia in patients with coccidioidal lesions documented elsewhere on their bodies.

Prevention

A number of efforts have been made to develop vaccines against *C immitis*. Inactivated vaccines have been prepared from mycelia or spherule extracts or from whole cells and administered to mice in oil-base adjuvants. Vaccination with formalin-killed spherules increases the LD50 for mice from 50 to 3000 arthrospores. Killed vaccines prepared from mycelia or spherules apparently do not prevent infection, although they do minimize dissemination and fungal replication and prolong survival of challenged animals. A large-scale placebo-controlled vaccine trial is now in progress in California. The vaccine is prepared from spherules of *C immitis*.

Attenuated strains have also been used as live vaccines. These have been protective in animal studies. However, attenuated strains are not eliminated by the host and persist for long periods in granulomas. Also, attenuation has proved somewhat unstable, with gradual reversion to virulent forms. Therefore, live vaccines are still considered unacceptable for use in humans.

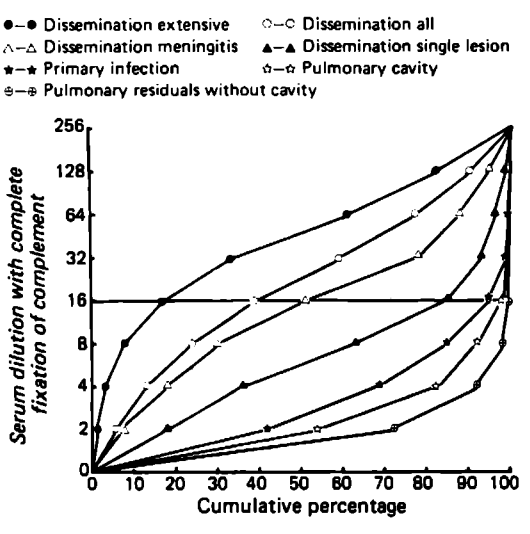

Figure 31–5. Relationship of coccidioidal complement-fixing antibody titer to various clinical forms of the disease. Note that the majority of patients with multi-lesion dissemination develop a complement fixation titer that exceeds 1:16. (Reproduced, with permission, from Smith CE et al: Pattern of 39,500 serologic tests in coccidioidomycosis. *JAMA* 1956;160:550. Copyright © 1956, American Medical Association.)

Treatment

Amphotericin B is the only drug with proved efficiency in coccidioidomycosis. Miconazole may be a useful alternative drug, but frequent posttreatment relapses may limit its value. Recent large trials with ketoconazole have suggested efficacy in some forms of the disease.

Because many patients with severe disease are refractory to amphotericin B and have defective cell-mediated immune responses to coccidioidin, a number of investigators have attempted immune reconstitution with transfer factor. Approximately 60 patients with a variety of lesions (including 11 with meningitis) have been treated. Immune conversions were dramatic, especially for MIF activity. Conversions were transient, requiring administration of more transfer factor to be sustained. Clinical improvement followed transfer factor administration in 60% of patients. These changes cannot be attributed to transfer factor with confidence at present, because (1) coccidioidomycosis is characterized by spontaneous remissions and relapses; (2) most patients were receiving concurrent amphotericin B; and (3) it is often difficult to distinguish subjective from objective improvement in this disease.

Complications & Prognosis

Erythema nodosum with or without erythema multiforme occurs in a varying proportion of patients with primary coccidioidomycosis. Such patients exhibit violently positive reactions to coccidioidin or spherulin skin tests. A negative coccidioidin skin test in a person with erythema nodosum or erythema multiforme is thought to rule out the diagnosis of coccidioidomycosis. The immunologic basis of the reaction is unknown.

Disseminated coccidioidomycosis should be considered an incurable illness in large numbers of patients, particularly in those who fail to recover coccidioidin reactivity following treatment. As in leprosy, therapy may have to be continued intermittently for life in patients whose immunologic status predisposes them to relapse. Ketoconazole may be particularly useful for maintenance of remission following a course of amphotericin B.

5. BRUCELLOSIS

Brucellosis is an acute or chronic illness manifested principally by chills, fever, and weakness. Occasionally, chronic relapsing febrile episodes occur, giving rise to the popular name "undulant fever." Brucellosis is endemic in many animal species; humans are infected incidentally, by either the oral or percutaneous route. Pregnant animals are especially susceptible to brucellosis and may abort. The growth of microorganisms in the placenta is apparently greatly stimulated by the local presence of erythritol in high concentration.

Once introduced into the body, brucellae quickly pass from lymph to regional nodes to bloodstream, where they are transported by PMNs and monocytes to sinusoids of the liver, spleen, bone marrow, and lymph nodes. The microorganisms multiply locally and are secondarily phagocytized by fixed macrophages of these reticuloendothelial tissues. Brucellae are relatively resistant to macrophage killing, and small numbers may survive the peak of heightened macrophage activity after an acute infection. It is not known whether brucellae survive because of innate resistance or because "incompetent" macrophages are unable to kill them. The subsequent appearance of clinical illness is apparently dependent upon host ability to restrain brucellar multiplication. In some cases the incubation period may be quite prolonged. The pathogenicity of *Brucella* organisms is at least partially related to differences in the composition of lipopolysaccharide in smooth virulent strains. It is possible that these may contribute to the slowed degranulation of specific granules in PMNs that have ingested *Brucella abortus*.

The usual histologic response to infection is granuloma formation, but in some tissues, especially spleen and bones, frank abscesses may develop. Abscesses are also more likely to occur when the infecting microorganism is *Brucella suis* or *Brucella melitensis; B abortus* more commonly produces granulomas. In mice, the formation of abscesses with *B melitensis* is associated with short-lived "acquired cellular resistance" and also with rapid elimination of all brucellae. On the other hand, *B abortus* produces granulomas, with evidence of prolonged hepatic infection.

The immunologic response to brucellar infection is both cellular and humoral. The importance of cell-mediated immunity is suggested by the successful use of viable attenuated *Brucella* species for vaccinating cattle and by the development of delayed skin test reactivity and in vitro lymphocyte transformation responses to *Brucella* antigens in persons who have had brucellosis. The *Brucella* skin test underwent a period of popularity in the USA but was withdrawn from commercial use because of nonspecificity. Protection conferred by vaccination for brucellosis, like that for salmonellosis, is only partial and can be overwhelmed by massive challenge doses.

Infection with *Brucella* species generates a vigorous antibody response. Although the antibody is not protective, rises in antibody titer may be used diagnostically. Both agglutinating and complement-fixing antibodies can be measured. Nearly all cases of acute brucellosis will show an agglutinin titer of 1:160 or greater within 3 weeks after infection; titers may persist at low levels for months or years, especially in patients with chronic infection. There is a problem of serologic cross-reaction with *Francisella tularensis, V cholerae*, and certain strains of *Yersinia enterocolitica*. In addition, a blocking factor has been described in some chronic cases. This results from the presence of IgA or IgG antibody, which produces spuriously negative agglutinins at low dilutions and, when added to high titer serum, depresses agglutinin levels. The prozone phenomenon can be avoided by carrying agglutinin titers out to a 1:320 dilution. Another way to serologically diagnose brucellosis is to mix the brucellae and the serum to be tested, wash the brucellae, and then add antihuman globulin. This equivalent of the Coombs test will cause brucellae to agglutinate even if a prozone phenomenon is present. Furthermore, a technique for passive hemagglutinating antibody is very sensitive and does not have the problem of cross-reaction with *Yersinia*. Serologic titers are very important in the diagnosis of brucellosis because the organisms are fastidious and require Castaneda's medium and CO_2 enrichment for optimal recovery from blood. Treatment (generally streptomycin plus tetracycline) may be complicated by the Jarisch-Herxheimer reaction. Relapses are common.

6. TULAREMIA

Francisella (Pasteurella) tularensis is a small gram-negative coccobacillus that requires special culture media for isolation. Infection occurs by inhalation or by invasion through abrasions in the skin. The organism is spread by contact with infected animals or by insect vectors such as ticks. Tularemia is an occupational hazard of trappers, sheepherders, mink ranchers, hunters, and butchers.

Tularemia may present in different ways depending upon the site of inoculation, but the most common clinical features are local ulcer formation and regional lymphadenopathy in association with symptoms of systemic febrile illness.

Host defense mechanisms are not fully understood. However, immunity is associated with the appearance of delayed hypersensitivity to tularemia skin test antigen. Also, in vitro responses of cell-mediated immunity can develop to the skin test antigen in sensitized persons. Immunity is associated with increased ability of rabbit macrophages to kill *F tularensis*. Inactivated vaccines are of no benefit, but a live attenuated strain has been successful in vaccinating humans and lower animals. This provides further evidence supporting the protective role of cell-mediated immunity. Finally, because the organisms are fastidious and highly infective, serologic responses are generally used to confirm the diagnosis. Tularemia agglutinins usually rise after the first 2 weeks of illness but may take much longer.

The test is relatively specific, with some cross-reactions with brucellae. As in brucellosis, there is no evidence that antibody formation is protective. Treatment is with streptomycin or gentamicin.

INFECTIOUS DISEASES CHARACTERIZED BY UNIQUE HOST-PARASITE RELATIONSHIPS

A number of infectious diseases do not fit easily into the preceding categories because of particularly unique aspects of their relationship to the host. Among these are syndromes resulting from infection with *Mycoplasma, Chlamydia,* and *Rickettsia* species and *Bordetella pertussis.*

Mycoplasma pneumoniae

Mycoplasmas, members of the class Mollicutes, are the smallest known free-living microorganisms and differ from bacteria in lacking cell walls. They have been recognized as etiologic agents of disease in humans, animals, insects, and plants. *Mycoplasma* species have unique morphologic, growth, and metabolic characteristics beyond the scope of this discussion.

The only *Mycoplasma* species with an unquestioned ability to regularly produce human infection is *Mycoplasma pneumoniae. Mycoplasma hominis* and *Ureaplasma urealyticum* may play a role in the pathogenesis of nongonococcal urethritis, pelvic inflammatory disease, postpartum fever, and low infant birth weight.

M pneumoniae, like *Bordetella pertussis,* appears to produce its major disease manifestations at the surface of epithelial cells. Studies in organ culture systems have indicated that infection is initiated by attachment of *M pneumoniae* to ciliated cells, followed by ciliostasis, loss of the cilia, and destruction of the mucosal epithelium. Unlike *Corynebacterium diphtheriae* and *V cholerae,* which also occupy an extraepithelial position, *M pneumoniae* produces no definite exotoxins. However, several nonhuman mycoplasmas are known to produce exotoxins.

Presumably, *M pneumoniae* is phagocytized and killed by leukocytes, although inhibition of macrophage function has been observed in vitro. The details of *Mycoplasma*-host interaction are outlined in Table 30–3.

Chlamydiae

These microorganisms are obligate intracellular parasites with a unique reproductive cycle. The infectious particle is a 0.3 μm structure termed the "elementary body" (EB); it is specialized for extracellular survival. Following endocytosis, a "reticulate body" (RB) is formed that multiplies intracellularly by binary fission to produce a large intracellular inclusion. Innumerable elementary bodies are secondarily formed that are then released by rupture of the host cell. Within macrophages, nonopsonized chlamydiae can apparently avoid lysosomal enzyme activity.

In addition to a unique developmental cycle, chlamydiae possess common morphologic characteristics and a common protoplasmic family antigen probably identical to the innermost (Re) core of the lipopolysaccharide of enteric bacteria (see page 544). Type-specific antigens are found in the cell wall. *Chlamydia trachomatis* and *Chlamydia psittaci* differ in 4 major respects: (1) *C trachomatis* is susceptible to sulfonamides, (2) *C trachomatis* forms rigid (as opposed to diffuse) microcolonies, (3) intracellular inclusions of *C trachomatis* are glycogen-positive and stainable with iodine, and (4) *C trachomatis* and *C psittaci* show little DNA homology.

Chlamydial diseases often run a protracted and relapsing course (especially trachoma). Chronicity of infection has led to the suggestion that chlamydial infection does not evoke an effective immune response. On the other hand, the host ordinarily restrains and localizes chlamydial disease without serious sequelae. Cell-mediated immune mechanisms appear to be important in chlamydial immunity. However, antibodies that combine with cell wall antigens of the parasite appear to prevent penetration of susceptible host cells, thus limiting the spread of infection.

Details of *Chlamydia*-host interaction are outlined in Table 30–3.

Rickettsiae

Rickettsial species are small gram-negative pleomorphic coccobacilli that function as obligate intracellular parasites. These organisms do not have flagella, pili, or attachment proteins that can be recognized morphologically. As is true for all bacteria, they possess a glycocalix (slime) layer that is easily sloughed; its pathogenic function is not known.

Rickettsiae of the typhus group *(Rickettsia prowazekii, Rickettsia typhi, Rickettsia canada)* possess common soluble antigens that cross-react in the complement fixation test. *Rickettsia* of the spotted fever group include *Rickettsia rickettsii* (Rocky Mountain spotted fever), *Rickettsia australis* (North Queensland tick typhus), *Rickettsia conorii* (boutonneuse fever), *Rickettsia akari* (rickettsialpox), and *Dermacentroxenus sibericus* (Siberian tick typhus). They not only share common antigens but are also characterized by the capacity to multiply in the nucleus as well as in the cytoplasm of infected cells. Among the other groups, only *R canada* can also invade the cell nucleus. The scrub typhus group contains only one species, *Rickettsia tsutsugamushi.* The etiologic agent of trench fever *(Rochalimaea quintana)* is no longer considered a *Rickettsia.*

Rickettsiae stimulate their own endocytosis and must quickly escape from the phagosome into the cytoplasm in order to survive. In contrast, *Coxiella burnetii,* the etiologic agent of Q fever, multiplies within phagolysosomes.

With the exception of *C burnetii,* members of the *Rickettsia* group share a number of important charac-

Table 30–3. Host-parasite relationships in mycoplasmal, chlamydial, and rickettsial infections.

Microorganism	Disease	Principal Manifestations	Pattern of Illness	Host-Parasite Interaction*	Serologic Tests	Comments
Mycoplasmas						
Mycoplasma pneumoniae	Primary atypical pneumonia	Pneumonia. Central nervous system and hepatic abnormalities, occasionally noted, may be due to antimycoplasmal antibody with shared antigens in brain and liver or to actual metastatic infection. Myringitis and Stevens-Johnson syndrome also reported.	Acute infection. Clinical disease may not occur until after several preceding asymptomatic infections. Thus, clinical illness may reflect the development of cellular hypersensitivity to the organism.	Extracellular. Attaches to neuraminic acid cell receptor via a specialized tip structure. Metabolic, cytopathic, and ciliostatic epithelial cell damage results, due partially to H_2O_2. Macrophages with attached mycoplasmas are immobile. Mycoplasmas evade phagocytosis perhaps because of shared antigens with host cell membranes. Organisms rapidly ingested and degraded in presence of specific antiserum. *Mycoplasma* species "patch and cap" on Thy 1$^+$ lymphocytes and other cells, then detach, carrying host antigens. Alteration of host antigen confirmation may lead to "autoimmune" response, supporting speculation that the pneumonia is largely an immune phenomenon.	CF (most commonly used). Many others available (indirect hemagglutination; immunofluorescence, etc). The sera of about 50% of patients with *M pneumoniae* infection contain antibodies to I antigen that cause nonspecific agglutination of human type O Rh-negative red blood cells at 4 °C. Cold agglutinins tend to be present as a function of the severity of mycoplasma infection.	*M pneumoniae* infection stimulates antibodies cross-reactive with red cell membrane and can cause an acute hemolytic response. Another cross-reactive antibody is able to agglutinate streptococci of species MG. Disease usually self-limited, but antibiotics (tetracycline, erythromycin) may shorten course without eliminating orgânisms. Inactivated vaccine is 70% effective in stimulating antibody and providing protection. Throat colonization is not affected. Immunization may sensitize, thus accentuating subsequent *M pneumoniae* infection.

*Antibody and CMI responses demonstrable, but protective role unclear. No apparent role for PMNs or opsonins, except as noted.

Abbreviations used: CMI, cell-mediated immunity; CF, complement fixation test; DIC, disseminated intravascular coagulation; EB, elementary body; PMN, polymorphonuclear neutrophil; RB, reticulate body.

Table 30–3 (cont'd). Host-parasite relationships in mycoplasmal, chlamydial, and rickettsial infections.

Chlamydiae						
Chlamydia psittaci	Psittacosis (ornithosis)	Pneumonia. Occasionally hepatitis, myocarditis, encephalitis, skin rash.	Broad spectrum of disease ranging from acute infection to latency. Immunity may be incomplete following infection; prolonged shedding of microorganisms sometimes occurs (especially in birds).	Obligate intracellular pathogen. EBs rapidly endocytosed by nonprofessional phagocytes and macrophages via interaction of a heat-labile surface factor with a protein cell surface receptor. Phagolysosomal fusion fails to occur in macrophage, and normal EB → RB → EB life cycle ensues in all cell types. In the presence of specific antibody, endocytosis by nonprofessional phagocytes is impaired; that by macrophages is enhanced; and phagolysosomal fusion occurs, apparently by antibody neutralization of the heat-labile surface factors. Chlamydial destruction ensues. Host cell damage may be partially attributable to poorly defined "chlamydial toxins."	CF (a group-specific test that measures antibodies to an antigenic determinant common to all chlamydiae [2-keto-3-deoxyoctanoic acid]). Cross-reacts with LGV and *Chlamydia trachomatis* D → K (see below). Rising CF titers assist in diagnosis, especially if titer > 1:16, but do not differentiate from LGV.	Frei skin test may be positive following psittacosis (see below).
Chlamydia trachomatis Serotypes A, B, Ba, C	Trachoma	Chronic follicular conjunctivitis, pannus, scarring.	Characterized by chronicity, latency, and relapse. Hypersensitivity response due to repeated exposure may play an important pathogenic role. Scarring may reflect "bystander" cell injury due to a vigorous immune response.	Obligate intracellular pathogen (predominantly epithelial cells of the eye). Infections limited to humans.	Microimmunofluorescence (micro-IF; measures specific antibodies to antigenic determinants in EB cell walls that are not detected by the CF test used for psittacosis/LGV). Can identify diverse serotypes of *C trachomatis* (A → K; see below) and can determine Ig class of the antibody.	The single greatest cause of blindness in the world. No skin test. No available vaccine (inactivated vaccine may accentuate subsequent infection). *Chlamydia* serotypes are identifiable within infected secretions and tissues by fluorescent-labeled monoclonal antibody staining methods.
Serotypes D → K	Nongonococcal (nonspecific) urethritis; NGU; NSU	Subacute purulent urethral discharge (males); asymptomatic carriage (females); purulent conjunctivitis and pneumonia (neonates).	Broad range; asymptomatic to acute.	Obligate intracellular pathogen (epithelial cells). Natural habitat is genitourinary tract.	Micro-IF (see above). CF titers commonly < 1:16 and cross-react with psittacosis/LGV.	A common cause of urethritis, salpingitis, perihepatitis, ophthalmia neonatorum, and neonatal pneumonia. Identical to the inclusion conjunctivitis agent.
Serotypes L1, L2, L3	Lymphogranuloma venereum (LGV)	Anogenital infection; lymphadenitis (buboes). Infection initially generalized.	Acute or chronic illness. May be considerable mucosal scarring. Latency common.	Obligate intracellular pathogen (epithelial cells and beyond). Much more invasive than other *C trachomatis* serotypes. Multiplies more readily in nonimmune macrophages than other serotypes.	CF (see psittacosis). Titers > 1:16 are most suggestive of LGV or psittacosis.	Sexually transmitted. Frei skin test (like CF antibody) measures a group antigen and may be positive in LGV or psittacosis. Frei test becomes positive well beyond changes in serologic tests; it is of little diagnostic value and is no longer available commercially.

Microorganism	Disease	Principal Manifestations	Pattern of Illness	Host-Parasite Interaction*	Serologic Tests	Comments
Typhus group						
Rickettsia prowazekii	Epidemic typhus	Headache, fever, rash.	Acute illness. Mild to severe, depending on specific microorganism. Immunity is usually long-lasting, with some exceptions: (1) Epidemic typhus may relapse 10–20 years after apparent recovery (Brill-Zinsser disease). Clinically milder. (No rash. No OX19 antibody titer rise.) (2) Trench fever is characterized by relapses.	Obligate intracellular pathogen (epithelial cells). Naturally pathogenic for arthropods. Bites → endothelial cell invasion → acute vasculitis → thrombosis (and DIC). Rickettsiae enter most cells by attaching to the cell membrane, then being endocytosed. The process is active for both host and parasite. Rickettsiae may actively enter macrophages but escape phagolysosomal fusion. If opsonic antibody is present, macrophages actively ingest rickettsiae → phagolysosomal formation → rickettsial death. Antibody does not affect endocytosis by nonprofessional phagocytes. Both antibody and cell-mediated immunity are important in host defense.	Weil-Felix, CF, Micro-IF	*All* can be diagnosed by CF test using yolk sac antigen or micro-IF, which is the best test available. *Many* can be diagnosed by their ability to stimulate formation of antibodies cross-reactive with strains of *Proteus vulgaris* (Weil-Felix reaction). *Examples:* Epidemic typhus → OX19 antibody. Scrub typhus → OXK antibody. Rocky Mountain spotted fever → antibody to OX19 and OX2. (Must eliminate any possibility of a concurrent *Proteus* infection.) No skin test. RMSF vaccine available, but efficacy uncertain.
Rickettsia typhi (mooseri)	Endemic typhus					
Rickettsia canada	?					
Spotted fever group						
Rickettsia rickettsii	Rocky Mountain spotted fever					
Rickettsia akari	Rickettsialpox					
Rickettsia conorii and others	Diverse names					
Scrub typhus group						
Rickettsia tsutsugamushi	Scrub typhus					
Q fever group						
Coxiella burnetii	Q fever	Headache, fever, pneumonia, hepatitis.	Acute or subacute illness.	Obligate intracellular pathogen. Can be acquired by inhalation.	CF	Vaccination effective but not practical.

*Antibody and CMI responses demonstrable, but protective role unclear. No apparent role for PMNs or opsonins, except as noted.

Abbreviations used: CMI, cell-mediated immunity; CF, complement fixation test; DIC, disseminated intravascular coagulation; EB, elementary body; PMN, polymorphonuclear neutrophil; RB, reticulate body.

teristics: (1) failure to survive in the environment; (2) dependence upon arthropods as vectors and reservoirs; (3) transmission by arthropod bites; and (4) multiplication in the endothelial cells of blood vessels, with production of vasculitis, thrombosis, hematogenous dissemination, and rash.

C burnetii is more often transmitted by the respiratory route, seldom involves biting arthropods, produces no rash, and is manifested principally by pneumonia.

The details of *Rickettsia*-host interaction are outlined in Table 30–3.

Bordetella pertussis

B pertussis is a short, ovoid, encapsulated, gram-negative rod which produces the clinical syndrome known as pertussis (whooping cough). Apparently other *Bordetella* species, as well as some viruses (especially adenoviruses), can also produce this syndrome. However, *B pertussis* infection is epidemic, whereas the others are sporadic. *B pertussis* acquisition is associated with a very high clinical attack rate; asymptomatic carriage is not common.

B pertussis possesses pili-like surface factors and 4 "phases" of in vitro growth (phases I and II are smooth and virulent; phases III and IV are rough and avirulent). In these and other respects, there is a striking resemblance to *N gonorrhoeae*. *B pertussis* multiplies only in association with the ciliated epithelium of the respiratory tract and does not invade epithelial cells. An important element of protection is surface antibody, probably IgA, which prevents adherence of *B pertussis* to epithelial cells. The microorganism is presumed to be killed by PMNs; its surface capsule is apparently not antiphagocytic. Many of the pathophysiologic manifestations of *B pertussis* infection have been attributed to one or more toxins that produce a necrotizing inflammatory response of the tracheobronchial mucous membrane and ciliary paralysis.

B pertussis is rich in a variety of potentially pathogenic factors (surface components, endotoxin, histamine-sensitizing factor, lymphocytosis-producing factor) the importance of which is unsettled in human infections. *B pertussis* elaborates a soluble, heat-stable, highly active adenylate cyclase that can be internalized by phagocytic cells, where it catalyzes the unregulated formation of cAMP. The resulting inhibition of superoxide generation, chemotaxis, and microbicidal activity may help to explain the high incidence of secondary bacterial infection that follows infection by *B pertussis*.

One of the most striking clinical accompaniments of pertussis is leukocytosis with an absolute lymphocytosis. This is accompanied by depletion of small lymphocytes in the thymus, spleen, and lymph nodes and reflects a failure of lymphocyte traffic back to the lymph nodes. Increased lymphocyte production is not considered to play a role. In vitro and animal studies indicate a variety of contradictory alterations of cell-mediated immune responses in pertussis (immune enhancement with increased or decreased tumor growth, increased or decreased infection susceptibility, etc). The precise role of cell-mediated immune mechanisms in pertussis is unclear. Successful immunization with a killed vaccine has reduced the threat of pertussis enormously in the USA. Presumably, this indicates that systemic humoral immune mechanisms may be more important than cell-mediated immunity in protection. The role of cell-mediated immunity in recovery from established infection is unsettled, however.

The use of pertussis vaccine in Great Britain is being seriously questioned because of a high incidence of neurologic sequelae.

INFECTIONS COMPLICATED BY DEPOSITION OF CIRCULATING IMMUNE COMPLEXES

Major Immunologic Features

- Infections often characterized by persistence or chronicity.
- Complications include glomerulonephritis, arthritis, or skin lesions.
- Immunologic manifestations may include hypergammaglobulinemia, cryoglobulinemia, hypocomplementemia.
- Immune complex deposition in target tissues triggers vasculitis.

General Considerations

The production of antigen-antibody complexes is probably quite common in a majority of infectious diseases, but symptoms rarely result from their presence. Ordinarily, they possess physicochemical characteristics that permit their asymptomatic clearance from the bloodstream by the reticuloendothelial system. In some circumstances, however, antigens and antibodies combine under conditions that predispose to their deposition in the walls of blood vessels. As a result of such deposition, complement is activated locally and results in the establishment of an inflammatory response through the release of soluble mediators, including chemotactic factors. The result of the inflammatory response is dependent upon the site of immune complex deposition; glomerulonephritis, arthritis, and skin lesions are common clinical manifestations of this process.

Table 30–4 deals with infectious diseases known to be complicated by immune complex deposition. There seems to be little question that more examples will be found in the near future.

The familiar "proteinuria of fever" is very likely a manifestation of subclinical glomerulonephritis in a broad variety of infectious diseases. Acute nephritis has been described in infectious mononucleosis, mumps, variola, varicella, adenovirus (type 7) and echovirus (type 9) infection, and following vaccination.

Erythema nodosum complicates many infectious diseases (leprosy, coccidioidomycosis, histoplasmo-

Table 30–4. Immune complex complications of infectious diseases.

Disease	Etiologic Agent	Immunologic Predisposition	Immunologic Complications	Comments
Infective endocarditis	Bacteria (*Streptococcus viridans; Staphylococcus aureus*, enterococci; many others). Fungi (*Candida albicans* and others). Miscellaneous (*Coxiella burnetii* and others; rare).	Continuous discharge of microorganisms or their antigens into the bloodstream. Infecting microorganisms protected from phagocytes by overlying fibrin network and by the avascular nature of cardiac valve tissue. High levels of rheumatoid factor may impair opsonic capacity of IgG which is specifically bound to microorganisms. Immunologic complications are directly proportionate to duration of infection (ie, more common in "subacute" than acute endocarditis).	Immune complex glomerulonephritis characterized by proteinuria, microscopic hematuria, and red cell casts in urine. Antibody, complement, and specific bacterial antigen present in renal vasculature. Nephritis may be focal or diffuse. Focal nephritis may occur in 50–90% with few sequelae. Diffuse nephritis occurs in < 10%; may produce uremia. Osler's nodes (painful nodular lesions in fingertips and elsewhere), Roth spots in ocular fundi (white center with surrounding hemorrhagic zone), petechiae, splinter hemorrhages beneath nails—all may reflect hypersensitivity angiitis characterized by intimal proliferation. "Embolic" complications (especially central nervous system) may often represent hypersensitivity angiitis. Splenomegaly and presence of circulating histiocytes (especially in earlobe blood) reflect chronic stimulation of reticuloendothelial system. Other immunologic accompaniments include hyperglobulinemia, rheumatoid factor, cryoproteinemia, and, perhaps, myocarditis and anemia.	Endocarditis is a disease of heart valves; valves may be destroyed by infection. Similar immunologic sequelae may be encountered in infected arteriovenous shunts, infective endarteritis, and infected central nervous system ventriculoatrial shunt devices (usually due to *S epidermidis*). Common feature to all is continuous discharge of microorganisms into blood. Treatment consists of antibiotics. Immunologic complications currently handled expectantly; corticosteroids may exacerbate infection and are usually contraindicated.
Viral hepatitis	Hepatitis B	Persistent antigenemia due to HBsAg stimulates production of anti-HBs; results in circulating immune complexes. If HBsAg is eliminated, resultant illness is similar to "one-shot serum sickness," with only transient clinical manifestations. If there is continual production of HBsAg, HBsAg-Ab complexes are continually present, resulting in an illness resembling chronic serum sickness in animals and expressed as a generalized necrotizing vasculitis. Why most patients who acquire HBsAg do not develop these syndromes but only subclinical or clinical hepatitis probably relates to poorly understood "host immune factors."	The syndrome equivalent to "one shot serum sickness" is characterized by migratory, additive, or simultaneous polyarthralgias and arthritis together with skin rash which is often urticarial. Hepatitis may be subclinical or associated with jaundice. Serum and joint complement may be decreased; cryoglobulins composed of HBsAg, anti-HBs, and complement may be found. Occasionally associated with glomerulonephritis. The syndrome equivalent to "chronic serum sickness" is characterized by multisystem disease with a prolonged course. Manifested by arthritis, renal disease, heart disease, etc, with the features of polyarteritis or hypersensitivity angiitis. Only by finding HBsAg can viral etiology be established. Otherwise, will be considered a "collagen vascular disease of unknown etiology." May need to test repeatedly for these viral factors. No apparent relationship between appearance of vasculitis and liver disease (if present).	"One-shot" syndrome often self-limited; usually preicteric. Hepatitis A may produce a similar transient syndrome. Necrotizing vasculitis syndrome is potentially life-threatening but may evolve as a chronic debilitating disorder requiring immunosuppressive therapy.
Poststreptococcal acute glomerulonephritis	*Streptococcus pyogenes*	May follow either pharyngeal or skin infection. Related to specific M types of *S pyogenes:* pharyngeal (types 1, 4, 12, and 18; perhaps 3, 6, and 25), skin (types 2, 31, 49, 52–55, 57, 60). Seven- to 14-day latent period between infection and onset of glomerulonephritis (similar to interval in experimental serum sickness).	Immune complex glomerulonephritis characterized by proteinuria, hematuria. May progress to hypertension, renal functional impairment, and edema of face and legs. All glomeruli diffusely involved. Antibody, complement deposited in renal vasculature. Hypocomplementemia during first 2–6 weeks of illness.	Usually heals; especially in children.

Table 30–4 (cont'd). Immune complex complications of infectious diseases.

Disease	Etiologic Agent	Immunologic Predisposition	Immunologic Complications	Comments
Quartan malaria	*Plasmodium malariae*	High level of parasitemia. High malaria antibody titers.	Immunoglobulins and complement in glomerular capillary walls. Nephrotic syndrome.	Poor response to corticosteroids. Most common in children 4–8 years of age.
Syphilis	*Treponema pallidum*	Congenital or secondary syphilis.	Nephrotic syndrome. "Hemorrhagic glomerulonephritis" has also been described.	Responds to penicillin.
Typhoid fever	*Salmonella typhi*	Persistent infection with prolonged bacteremia.	Antibody, complement, and *Salmonella* "Vi" antigen in glomerular capillary walls → glomerulonephritis.	Probably a common complication of typhoid fever but rarely appreciated because renal biopsies are seldom done (or justified).
Leprosy	*Mycobacterium leprae*	Erythema nodosum leprosum (see description elsewhere in chapter).		

sis, tuberculosis, and streptococcal infection, to name but a few). It is likely that the cause of this complication will prove to be immune complex deposition (as does seem to be the case in leprosy) when appropriate studies are performed.

The natural history of many virus infections (and of leptospirosis) is biphasic, and the development of gross clinical phenomena is often preceded by a nonspecific febrile illness. Whereas the nonspecific illness is often the direct result of the virus infection, the more specific manifestations of the disease such as the exanthems are probably due to the presence of immune complexes formed by virus and antibody in the circulation. The mechanism of development of the rash may be similar to that in serum sickness.

In a simple exanthematous disease such as rubella, other signs due to circulating immune complexes, such as arthritis, may be seen occasionally.

THE SPECTRUM OF HOST-VIRUS IMMUNOLOGIC RELATIONSHIPS

Major Immunologic Features

- Disease may be acute, latent, chronic, or delayed ("slow virus") in onset.
- Altered disease presentation may result from prior immunization with inactivated virus.

General Considerations

Viral diseases can be divided into 2 main categories with respect to the ultimate fate of the virus in the tissues:

(1) Virus may be eliminated from the body: This is the pattern encountered in the vast majority of human viral infections, ranging from smallpox to influenza to poliomyelitis. Appropriate secretory and systemic, cellular and humoral immune defenses are mobilized, and the virus is dealt with appropriately. Subsequent immunity is solid and usually persists for life. (Some investigators regard the firm persistence of immunity as evidence that the virus may continue to be sequestered in the tissues for life, serving to chronically boost the host immunologic response. Even if this is the case, the viruses in this group never manifest themselves clinically beyond the initial acute illness.)

(2) Virus may persist in the body and produce disease: An uneasy immunologic balance between virus and host may obtain in certain viral infections, giving rise to patterns of latency, chronicity, or extremely delayed ("slow") onset. Examples of acute and persistent host-virus relationships are outlined in Table 30–5.

A third category of viral illness may be encountered in patients who have previously received certain inactivated vaccines. Here, vaccine-modified host defenses accentuate to a greater or lesser degree the characteristics of subsequent natural infection, while failing to provide adequate protection. Examples of these vaccine-activated illnesses are discussed briefly below.

Measles

Recipients of formaldehyde-inactivated measles vaccine, upon subsequent natural exposure to measles, may develop a hyperacute "atypical measles" syndrome characterized by an inordinate febrile response, pneumonia with pleural effusion, and a severe hemorrhagic rash in atypical distribution. Severe local skin reactions may also occur when previous recipients of inactivated measles vaccine receive an inoculation with live vaccine.

It has been suggested that the prior vaccination results in augmentation of delayed hypersensitivity to measles virus. Alternatively, there is evidence that "atypical measles" may reflect immune complex deposition. According to the latter explanation, inactivated vaccine produces serum IgG antibody titers, which then wane. Upon subsequent exposure to the live virus, a marked anamnestic IgG response occurs, producing antibody that complexes with viral antigen

Table 30–5. The spectrum of host-virus immunologic relationships.*

Fate of Virus in Tissues	Type of Infection	Characteristic Host-Virus Relationships	Specific Examples	Comments
Eliminated	Acute	Short incubation periods (2 days to 2 or 3 weeks). Virus recoverable before but not after onset of disease. Recovery common. Recovered host is immune to same or closely related viruses.	Smallpox (high clinical attack rate; usually results in disease). Poliomyelitis (rarely results in clinical disease). Innumerable others.	The vast majority of the acute viral infections of humans fall into this immunologic category. Immunity is solid and persistent.
Not eliminated; persists in host tissues for months, years, or a lifetime	Latent	Acute primary infection followed by recovery and subsequent relapses and remissions. Virus recoverable during primary and relapsing phases of infection; not recoverable from target tissues during remissions. Host immune response demonstrable but ineffective in preventing relapse.	Herpes simplex ("fever blisters"). Varicella-zoster ("shingles").	These viruses apparently persist in nerve ganglions during periods between attacks. Humoral antibody is demonstrable but not protective. Cell-mediated immunity is critical but not sufficient to prevent relapses. Immunosuppressive diseases or drugs may permit dissemination of these otherwise well-localized recurrent illnesses.
	Chronic	Variable incubation period, outcome, and course. Virus persists and is regularly recoverable. Host immune response demonstrable but does not influence pattern of disease.	Congenital rubella syndrome.	Infection of fetus occurs during second or third month of gestation. Persistent viral infection produces congenital abnormalities and vasculitis. Brisk IgM antibody response; depressed cell-mediated immunity.
			Cytomegalovirus infection.	May produce mental retardation, hepatitis when acquired in utero. Hepatitis, rash, and "mononucleosis-like" syndrome in older persons. Commonly asymptomatic but becomes apparent (variably symptomatic) in the presence of immunosuppression. High antibody titers are not protective.
			Hepatitis B infection.	Eighty-five percent of patients with acute hepatitis show HBsAg for 1–13 weeks; 5% for up to 6 months; some carry HBsAg chronically. Persistence of carriage is associated with suppression of T cell function.
	Slow	Incubation period of months to years. Relentless progress and lethal course of disease.	Kuru; Creutzfeldt-Jakob disease (humans); scrapie (sheep); transmissible mink encephalopathy (mink).	Progressive demyelinating syndromes characterized by "subacute spongiform encephalopathy." Kuru and Creutzfeldt-Jakob disease are transmissible to chimpanzees. Virus (or viroid) not identified.
			Progressive multifocal leukoencephalopathy.	Due to a conventional virus (SV40 or similar DNA virus) which may constitute part of the "normal brain flora." Disease emerges under immunosuppression. Virus recoverable throughout course of disease. Minimal inflammation or other evidence of host immune response.
			Subacute sclerosing panencephalitis (SSPE) (Dawson's inclusion body encephalitis).	Due to a conventional virus (measles virus) which produces persistent infection. Abnormally high titers of measles antibody present (depressed cell-mediated immunity). High IgG levels. Epidemiologic evidence suggests very early measles infection (< 2–3 years of age) with manifestations of SSPE in early teens (= "slow"). Might be classified with the "chronic" group.

*Adapted from Youmans GP, Paterson PY, Somers HM (editors): *The Biological and Clinical Basis of Infectious Diseases,* 2nd ed. Saunders, 1980.

Table 30–6. Opportunistic infections.

Immunologic Setting	Commonly Associated Pathogens	Comments
Hypogammaglobulinemia (congenital or acquired)	Pyogenic extracellular bacteria. Some viruses.	Loss of opsonic or neutralizing antibody. (*S pneumoniae* infection is especially common in multiple myeloma.)
Granulocytopenia (< 1000 circulating granulocytes/μL; often due to cancer chemotherapy, bone marrow failure)	Pyogenic extracellular bacteria. Gram-negative enteric bacteria (*E coli*, etc). Environmental gram-negative bacteria (*P aeruginosa; S marcescens*). *Candida albicans*.	Inadequate circulating phagocytic cells (qualitative granulocyte defects give rise to similar infections).
Depressed cell-mediated immunity due to basic illness (Hodgkin's disease) or drug therapy (cancer chemotherapy; organ transplantation)	Intracellular pathogens (*M tuberculosis, H capsulatum*, etc). Rare "unusual" pathogens (*N asteroides; L monocytogenes, Legionella pneumophila, Pneumocystis carinii, Toxoplasma gondii*). DNA-type viruses (varicella-zoster, cytomegalovirus, wart virus, progressive multifocal leukoencephalopathy).	Defective lymphocyte function. (*T gondii* is actually a very common pathogen; immunosuppression permits reactivation of quiescent cysts, especially in the brain). (*P carinii* is a protozoan parasite of the pulmonary alveolar space, where it replicates in a proteinaceous coagulum. There are no useful immunologic tests for its presence. It produces an alveolar capillary block [arterial shunt] syndrome.)
Hemolysis (chronic, severe) (especially with sickle cell disease, malaria, bartonellosis)	Salmonellosis with bacteremia and secondary local abscess formation.	*Salmonella* species may compete with red cell breakdown products for macrophage membrane receptor sites, resulting in impaired phagocytosis. In sickle cell disease, *Salmonella* may infect bone infarcts, leading to high incidence of *Salmonella* osteomyelitis.
Splenectomy	Pyogenic encapsulated extracellular bacteria (especially *S pneumoniae*). Rarely, malaria, piroplasmosis (babesiosis).	Fulminating and rapidly fatal bacterial infection, often complicated by disseminated intravascular coagulation. Risk of infection far greater when there is an underlying disease of the reticuloendothelial system or when the patient is very young (eg, adolescent splenectomy for trauma carries less risk than childhood splenectomy for thalassemia). Spleen is critically important in the early control of bacteremia, prior to the synthesis of specific opsonic antibody. Impaired synthesis of IgM, tuftsin, and complement shunt pathway components may follow splenectomy. Loss of "pitting" function for malaria and similar protozoa.
Foreign bodies (intravascular, intra-articular, etc)	Pyogenic extracellular bacteria (especially *S aureus* and *S epidermidis*). *Candida albicans* (especially during total parenteral nutrition, hyperalimentation).	Direct vascular portal from the skin (intravascular catheters). High *Candida* incidence in hyperalimentation may reflect glucose-rich material infused. Arteriovenous shunts (as from dialysis) may predispose to infective endocarditis by changing flow characteristics across heart valves, leading to predisposition to incidental infection.
Gastrectomy	Pulmonary tuberculosis. *Salmonella* gastroenteritis. Cholera.	Malnutrition due to excessive stomach removal (tuberculosis reactivation). Loss of gastric acidity barrier (salmonellosis and cholera).
Acquired immunodeficiency syndrome (AIDS)	*Pneumocystis carinii, Toxoplasma gondii, Cryptosporidium* sp (protozoa), *Cryptococcus neoformans, Candida albicans* (mucosal), *Mycobacterium avium-intracellulare*, herpes simplex, cytomegalovirus; herpes zoster (DNA viruses).	Etiologic agent presumed "a virus"; candidates include cytomegalovirus, retroviruses. Dominant host population includes male homosexuals, intravenous drug abusers, hemophiliacs, Haitians, and their sexual contacts. Mode of disease transmission appears to be blood and secretions (similar to hepatitis B). Profound lymphopenia with absolute depression of helper T cell population.

and precipitates a subsequent Arthus response in the respiratory tract and skin.

Respiratory Syncytial Virus

Recipients of formaldehyde-treated, alum-precipitated respiratory syncytial virus (RSV) vaccine may develop clinically typical but much more severe illness upon subsequent natural exposure to the virus. Bronchiolitis and pneumonia, in particular, are accentuated. Again, augmented delayed hypersensitivity or Arthus-type immune complex deposition in the bronchiolar walls has been held responsible.

Immunologic exacerbation of infection by prior vaccination is not unique to viral diseases. Local ocular immunization with *Chlamydia trachomatis* vaccine has been shown to predispose to more intense ocular infection upon subsequent challenge with a wild type heterologous trachoma strain. Immunity to the homologous strain, in contrast, is intact. Delayed hypersensitivity is an important immune mechanism in trachoma and is presumably intensified by prior immunization. Similarly, *M pneumoniae* infection is intensified by prior immunization, but only in those patients who fail to develop growth-inhibitory *Mycoplasma* antibody. These patients appear to be sensitized by the prior vaccination. This phenomenon is of

particular interest because the occurrence of *M pneumoniae* pneumonia (typically in young adults) is considered to reflect an immune response to an agent encountered frequently (and asymptomatically) throughout early childhood.

OPPORTUNISTIC INFECTIONS

These diseases are the consequence of defective functioning of the normal immune system, predisposing the patient to infections characteristic for the compromised immune function. The infecting pathogen may be either common or rare, in the latter case emerging only under circumstances of defective immunity. Restoration of normal immune status may be of greater importance in recovery than antimicrobial drug therapy.

Nothing is more revelatory of the normal functional capacity of the immune system than the infectious diseases that result when immunity is suppressed. In a sense, every infectious disease represents an opportunistic infection. (For example, pneumococcal pneumonia occurs because of the opportunity afforded by aspiration of secretions and the absence of circulating type-specific opsonic antibody.)

The diseases summarized in Table 30–6, however, result when there is a more flagrant disruption of the functional capacity of the immune system. There is no attempt to list every potential opportunistic pathogen; major relationships are stressed.

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31 Endocrine Diseases

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Some of the best-studied examples of organ-specific autoimmune disease are found among the endocrine disorders, in which the target antigen of the immunologic response is unique for the affected organ. Such antigens are often associated with the unique function of the organ. Organs of internal secretion, with their highly specialized physiologic functions, possess distinctive organ-specific antigens (Table 31–1).

CHRONIC THYROIDITIS

Major Immunologic Features

- Produced experimentally by injection of thyroglobulin in adjuvants.
- Thyroid function tests may be elevated, depressed, or normal.
- Autoantibodies to thyroglobulin or thyroid microsomes (or both) present.
- Self-limited or responsive to thyroid hormone treatment.

General Considerations

The thyroid gland is made up of a series of saclike follicles lined with cuboidal epithelium. Within the follicles is found the homogeneously stained colloid, the principal constituent of which is a glycoprotein, thyroglobulin. This high-molecular-weight protein (about 650,000) contains iodinated amino acids, ie, mono- and diiodotyrosine, triiodothyronine (T_3), and thyroxine (T_4). The latter 2 amino acids are the active thyroid hormones. Similar proportions of each hormone (99.9% T_4, 99.5% T_3) are bound to plasma proteins, but the binding affinity for T_3 is much lower; its distribution volume is greater; and a greater proportion of the extrathyroidal hormone is located within cells. The synthesis of thyroglobulin in the follicle-lining cells is usually balanced by its resorption and splitting by thyroid proteolytic enzymes or cathepsins. Thyroglobulin is essentially a molecular storage form of the thyroid hormones. Thyroglobulin breakdown is enhanced by one of the peptides secreted by the anterior pituitary, termed thyroid-stimulating hormone (TSH) or thyrotropin, and decreased by iodide.

It was formerly believed that thyroglobulin is anatomically sequestered from the vascular and lymphatic pathways. More recent information indicates that low levels of thyroglobulin are found in the lymphatics draining the thyroid and in the blood-

Table 31–1. Endocrine diseases with autoimmune phenomena.

Disease	Antigen(s)	Methods for Detection of Antibody
Chronic thyroiditis and primary hypothyroidism	Thyroglobulin	Precipitation Hemagglutination Radioimmunoassay ELISA Immunofluorescence
	Microsomes of thyroid epithelium	Complement fixation Immunofluorescence ELISA Hemagglutination
	Membranes	Cytotoxicity
Hyperthyroidism (Graves' disease)	TSH receptor of thyroid cell surface	Radioreceptor assay In vitro thyroid stimulation assays
Adrenal insufficiency (Addison's disease)	Microsomes of adrenal cortex Heat-stable antigen of adrenal cortex Steroid-producing cells of adrenal, ovary, testis, and placenta	Immunofluorescence Complement fixation Precipitation
Primary hypoparathyroidism	Oxyphil cells Chief cells	Immunofluorescence
Diabetes mellitus	Beta* cells of pancreatic islets	Immunofluorescence
Ovarian failure	Cells of theca interna of corpus luteum	Immunofluorescence

*See note on p 593.

stream. Plasma thyroglobulin is elevated in many cases of hypertension and some cases of thyroid carcinoma and subacute thyroiditis; thyroglobulin concentrations are less often elevated in toxic nodular goiter and lymphocytic thyroiditis. Injection of an animal's own thyroglobulin, combined with certain adjuvants, elicits production of specific autoantibodies. If given with Freund's complete adjuvant (an emulsion with mineral oil, acid-fast microorganisms, and an emulsifying agent) or with bacterial lipopolysaccharide, thyroglobulin injections elicit lymphocytic infiltration of the thyroid gland of the immunized animal.

Injection of foreign thyroglobulins also causes thyroiditis without the need for adjuvants. Thyroglobulin molecules of the same species can be rendered antigenic by inserting foreign chemical determinants such as arsenilic or sulfanilic groups. These apparently provide immunologic handles similar to those supplied by the foreign determinants of cross-reacting thyroglobulins. It is also possible to make thyroglobulin antigenic by incomplete proteolytic digestion. Perhaps this procedure exposes unfamiliar sites of the molecule that act like foreign determinants.

The immunologic reaction to thyroglobulin is determined in part by the innate, genetically determined responsiveness of the injected animal. For example, among mice, some inbred strains are excellent responders to their own thyroglobulin while other strains are very poor responders. It is probable that several genes are involved in determining the response. The ability to produce circulating antibody to thyroglobulin can sometimes be separated from the ability to develop autoimmune lesions in the thyroid. One of the main genes controlling recognition is linked to the major histocompatibility (H-2) complex of the mouse and is probably an Ir gene coding response to a particular determinant or small number of determinants on the thyroglobulin molecule.

Some animals have a strong tendency to develop thyroiditis spontaneously, such as certain strains of beagle dogs and of rats. A closed colony of chickens called OS (for obese strain) has been established by selective breeding, and these birds have severe thyroid inflammation with typical clinical and biochemical evidence of thyroid failure. Study of these hereditary models of thyroiditis provides valuable clues about the genetic predisposition to autoimmune disease of the thyroid in humans. Among OS chickens, 3 basically different types of genetic defects seem to predispose the animals to the development of thyroiditis. One gene is linked to the major histocompatibility complex and is probably similar to the Ir genes of the mouse. A second gene controls the maturation of the thymus, especially the emigration of helper and suppressor T cells. Finally, a third gene regulates thyroid function The OS thyroid takes up abnormally large amounts of iodine.

Immunologic Pathogenesis

The similarity of chronic thyroiditis in the human to experimentally induced or hereditary thyroiditis in animals is striking. The principal antigen is thyroglobulin. Under experimental conditions, this purified protein can be shown to be autoantigenic. Immunization results in production of autoantibodies reactive with antigen from the immunized animal itself. Lesions arise simultaneously in the thyroid gland of the animal, and these lesions are quite similar to those of the human disease. Human chronic thyroiditis fulfills these minimal criteria for diseases of autoimmune origin (Table 31–2).

Defining the precise pathogenic mechanisms of thyroiditis has proved to be difficult. In some species, such as the rabbit, it has been possible to transfer the disease from immunized to normal animals by injecting large quantities of antibody-containing serum.

In other species, such as the guinea pig or rat, thyroiditis has thus far been transferred only with living, histocompatible lymph node cells. The histologic features of thyroiditis are more suggestive of cell-mediated than of antibody-mediated immunologic reactions. It is possible to demonstrate delayed hypersensitivity by skin tests in immunized guinea pigs and rats. In vitro indicators of cell-mediated immunity such as production of macrophage migration inhibitory factor (MIF) and lymphocyte transformation have also been shown. T cells from mice with experimentally induced thyroiditis are directly cytotoxic to thyroid epithelial cells grown in culture.

In the test tube, it can be shown that normal lymphocytes of the human can cooperate with thyroid antibody to damage thyroid cells or lyse carrier cells coated with thyroglobulin or thyroid microsomes. These findings support the view that antibody-dependent cell-mediated lymphotoxicity is responsible for the induction of pathologic changes in the thyroid. Immune complexes have also been found in the thyroid basement membrane of a few patients with thyroiditis. Complexes of appropriate size may adhere to the stroma of the thyroid gland, activate complement, and provoke inflammation. Obviously, more work must be done to define the pathogenetic mechanisms in thyroiditis both in humans and in experimental animals. Lymphocyte suspensions from the infiltrated thyroid glands of thyroiditis patients contain fewer total T cells and T cells of the helper/inducer (CD4) subset than lymphocytes from peripheral blood. There is an increase in the percentage of B cells, corresponding to the presence of germinal centers in the glands.

Table 31–2. Criteria for establishing the autoimmune etiology of an organ-specific human disease.*

(1) Autoantibodies reactive at body temperature, or evidence of cell-mediated immunity
(2) Isolation and purification of the organ-specific antigens
(3) Production of autoantibodies to analogous antigen in experimental animals
(4) Development of similar lesions in autosensitized animals

*Modified from Witebsky E et al: Chronic thyroiditis and autoimmunization. *JAMA* 1957;164:1439.

Lymphocytes isolated from these tissues synthesize thyroid autoantibody in culture in the absence of mitogenic triggering. B lymphocytes from the blood of patients with autoimmune thyroiditis produce thyroglobulin antibody only when stimulated with pokeweed mitogen combined with thyroglobulin antigen. These findings provide evidence for the role of the thyroid as a major site of autoantibody synthesis, but antibodies to thyroglobulin and thyroid microsomes are also produced in the deep cervical lymph nodes that drain the thyroid lymphatics and in the bone marrow. Because of its large size, the latter organ may, in fact, be the most important site for autoantibody synthesis.

Most studies suggest that suppressor cell function in patients with thyroiditis is normal. Using a modified migration-inhibition test with purified T lymphocytes as the migrating cells, Volpé and his colleagues described antigen-specific suppression. This mechanism seems to be defective in patients with autoimmune thyroid disease.

Clinical Features

Chronic lymphocytic thyroiditis is most common in the age group from 30 to 60 years, although juvenile thyroiditis may cause sporadic goiter in children and adolescents. The female-to-male ratio is about 5:1, an indication that sex hormones may influence expression of the disease. The incidence of thyroiditis in monozygotic twins is about 6 times greater than in dizygotic twins of the same sex, suggesting a strong genetic predisposition. A number of studies have pointed to a relationship of thyroiditis and chromosomal aberrations, especially Down's syndrome. Although unpredictable in its occurrence, the disease is found more frequently in persons who have relatives with a thyroid disorder, such as thyroiditis, hyperthyroidism, or myxedema. The relatives may have thyroid antibodies without overt disease or may have autoimmune disease of another endocrine gland, pernicious anemia, or atrophic gastritis. This immunologic overlap among the organ-specific autoimmune disorders has been taken as tentative evidence that there is some genetic fault in the immunologic regulatory mechanisms that normally control self-reactive lymphocytes.

The subacute and "silent" (painless) forms of thyroiditis sometimes occur in small outbreaks following viral infections. Mumps has been reported to predispose to thyroiditis. It may be that respiratory tract infections can trigger autoimmunity to thyroid, but this relationship has not been clearly demonstrated in cases of chronic thyroiditis. In some cases, postpartum thyroiditis may be autoimmune in nature. There may be lymphocytic infiltration of the thyroid and serologic evidence of thyroid autoantibodies. Some patients have repeated postpartum episodes of thyroiditis and show increasing antibody titers as the disease progresses.

The clinical features of chronic thyroiditis in humans are relatively mild. The thyroid gland may be diffusely enlarged to produce a goiter. It is usually firm to hard in consistency, only rarely tender, and smooth or scalloped without distinct nodules. Severe symptoms of neck pain with upward radiation may be present.

Most chronic thyroiditis patients are clinically euthyroid even though uptake of radioactive iodine by the thyroid is high. About 20% are hypothyroid when first seen, and occasionally hyperthyroidism is present initially in the disease. During the later phases of chronic thyroiditis, patients may develop signs of diminished thyroid function, suggesting failure of regeneration of the epithelial cells. The skin is dry and the hair coarse, and myxedema is sometimes present. These patients show lowered values of circulating thyroid hormones, decreased thyroidal radioiodine uptake, high serum TSH levels, and high serum cholesterol levels. Their basal metabolic rates are low.

Immunologic Diagnosis

In humans, as in animals, thyroglobulin is the major autoantigen of the thyroid (Table 31–1). Approximately 60–75% of patients with various forms of chronic thyroiditis (depending upon the histologic type) show positive reactions in an indirect hemagglutination test (Table 31–3). About a third of these patients have elevated hemagglutination titers of 600–1000 or more. Titers in this elevated range are strongly indicative of autoimmune thyroiditis or a related autoimmune process. Patients with primary adult myxedema may also demonstrate high titers of antibody to thyroglobulin in the same high prevalence as thyroiditis (Table 31–3). About a third of patients with hyperthyroidism due to Graves' disease and one-fourth of patients with carcinoma of the thyroid (especially papillary adenocarcinoma) have antibodies to thyroglobulin, usually with titers of less than 600 (Table 31–3). In many cases of hyperthyroidism or of thyroid cancer, localized lymphocytic infiltrates are found. The presence of antibodies in these 2 diseases may be indicative of a secondary process of autoimmunization.

Table 31–3. Incidence of thyroglobulin autoantibodies in various thyroid diseases and controls (tanned cell hemagglutination method).

Histologic Diagnosis	Approximate Percentage of Positive Reactions
Thyroiditis	
Acute	10%
Subacute (granulomatous or de Quervain's)	35%
Fibrotic (Riedel's)	50%
Chronic	
Lymphocytic	60%
Fibrous	75%
Mixed (nonspecific)	75%
Primary hypothyroidism (adult myxedema)	75%
Graves' disease (hyperthyroidism; thyrotoxicosis)	40%
Carcinoma	25%
Control hospital population	5%

Autoantibodies to thyroglobulin may be found in a few other diseases. Patients with pernicious anemia or atrophic gastritis frequently have antibodies to thyroid antigens. Patients with idiopathic adrenal insufficiency or parathyroid failure sometimes have autoantibodies to thyroid. In Sjögren's syndrome, autoantibodies to thyroglobulin are sometimes found in the same high titers.

Autoantibodies to thyroglobulin are reported in about 3–18% of individuals with no clinical evidence of thyroid disease. This figure depends to a great extent upon the age and sex of the group being sampled. For example, the normal reactor group is much higher if made up of female subjects 40–60 years of age. It is quite plausible that the appearance of autoantibodies in these individuals actually signifies a subclinical focal thyroiditis, which has been frequently reported in middle-aged women. Almost one-fifth of older women were reported to have lymphocytic thyroiditis in one postmortem study.

The autoantibodies to thyroglobulin localize in the colloid of methanol-fixed sections, producing a floccular appearance (Fig 31–1). The antibodies may be found in any of the major immunoglobulin classes, IgG, IgM, or IgA. However, they do not fix complement. By isoelectric focusing, they are highly dispersed, indicating their polyclonal origin.

Sera from patients with thyroiditis may react with a different antigen present in thyroid cells. Referred to as **microsomal antigen,** it can be localized in the cytoplasm of the thyroid epithelial cell (Fig 31–2). About 70% of patients with thyroiditis have this antibody. Since the patients with antibodies to thyroid microsomes do not always correspond to those with thyroglobulin antibodies, the sum of patients with either antibody is about 97%.

A third type of antibody is occasionally found. This type reacts with the colloid in a homogeneous pattern but cannot be absorbed with thyroglobulin. The reaction is attributed to a second colloid antigen. The antigen, which is not well characterized, is a noniodinated colloid protein (Table 31–1).

When serum of certain thyroiditis patients is mixed with viable thyroid cell suspensions in the presence of complement, it will damage the thyroid cells. The antigen is a component of the thyroid epithelial cell membrane.

Positive assays for TSH receptor antibodies are encountered in 10–15% of patients with lymphocytic thyroiditis. This is particularly apt to be the case where there is an initial hyperthyroid phase. It is possible that in some instances where radioreceptor techniques are used, the antibodies measured are in fact receptor-blocking rather than receptor-stimulating.

Differential Diagnosis

Chronic lymphocytic thyroiditis must be distinguished from nontoxic goiter, subacute thyroiditis, Graves' disease, and several types of cancer of the thyroid. The first distinction can be difficult, but nontoxic goiter is usually less firm than thyroiditis, and the presence of distinct nodularity favors the diagnosis of nontoxic goiter. In adults, high titers of thyroid autoantibodies will favor a diagnosis of thyroiditis. In adolescents, however, thyroiditis may be present in the absence of high antibody titers. Evidence of hypothyroidism favors a diagnosis of thyroiditis over that of either nontoxic goiter or thyroid cancer. Thyroid cancer would be suggested by the finding of very firm or hard nodules, adherence to skin or underlying structures, hoarseness due to invasion of the recurrent laryngeal nerve, and regional lymphadenopathy, all of which are unusual signs in thyroiditis and nontoxic goiter. A thyroid scintiscan is of critical importance in the evaluation of the nodular thyroid. Areas of nonfunction ("cold" nodules) are unusual in thyroiditis as compared to nontoxic goiter and cancer. A needle biopsy can be done if thyroiditis is suspected but should not be relied upon if cancer is more likely. In the latter instance, if a tissue diagnosis is desired, excisional biopsy is necessary.

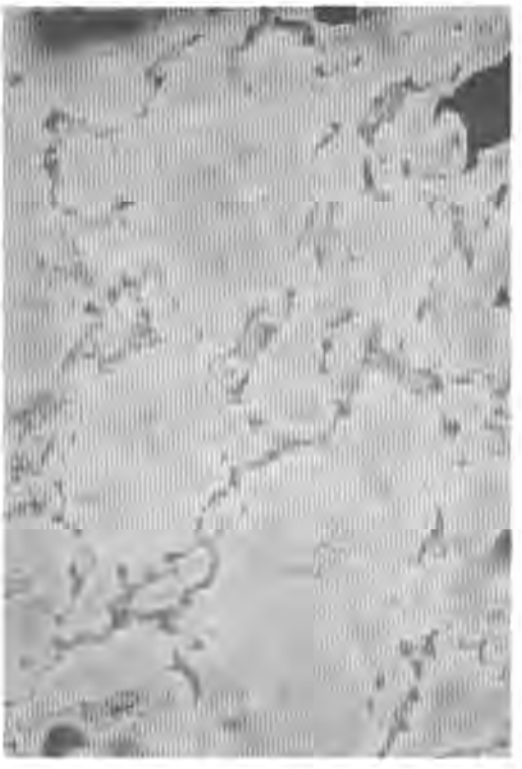

Figure 31–1. Immunofluorescent staining of thyroglobulin. Monkey thyroid tissue was frozen rapidly, thin-sectioned with a cryostat (4 μm), and fixed in methanol at 56 °C for 10 minutes. It was covered with serum from a patient with chronic thyroiditis (diluted 1:10), washed, covered with fluorescein-labeled anti-human globulin goat serum, washed, and examined with the ultraviolet microscope. A floccular pattern of fluorescence is seen within the thyroid follicles, indicating staining of the colloid. (Original magnification ×180.)

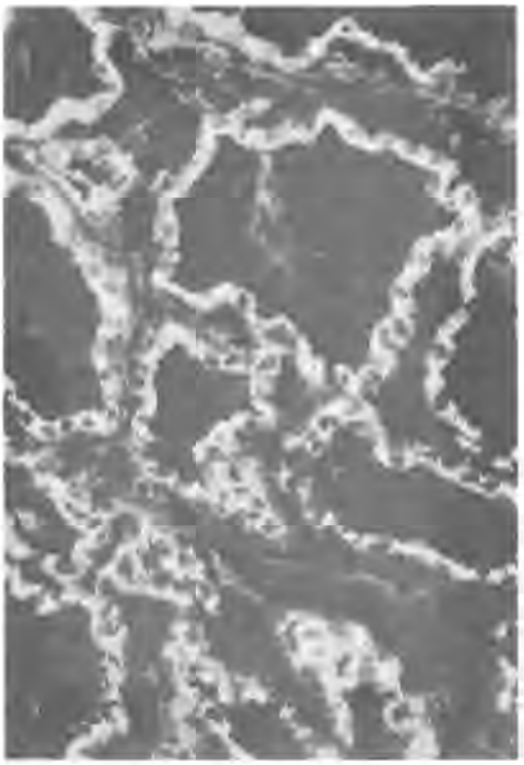

Figure 31–2. Immunofluorescent staining of thyroid microsomal antigen. Monkey thyroid tissue was prepared in the same manner as that described for Fig 31–1 except that it was not fixed in methanol. Immunofluorescent staining is seen in the cytoplasm of the thyroid epithelial cells lining the follicles. Note that the nuclei of the epithelial cells and the interstitial cells are unstained. (Original magnification ×180.)

Patients with subacute thyroiditis may complain of sore throat occurring coincident with or before the onset of thyroiditis. Perhaps the most significant statement a patient with subacute thyroiditis makes is that the throat is "sore on the outside rather than the inside." In contrast to chronic thyroiditis, the goiter of subacute thyroiditis may be accompanied by early elevations of serum thyroxine, probably as a result of release of hormone from the damaged gland, but these values usually return to normal after an intervening hypothyroid phase.

Acute thyroiditis is usually due to infection with pyogenic or mycobacterial microorganisms. It is occasionally associated with autoantibody production; and, although lymphocytic infiltration may occur, histologic resolution of the disease is usually rapid and complete.

Histologically, several forms of thyroiditis can be differentiated (Table 31–3). Some thyroids show granulomas containing typical giant cells and epithelioid cells. This form of thyroiditis, referred to as de Quervain's type, corresponds to the subacute disease, the most common type following viral infection. The patients show systemic evidence of inflammation, such as fever, a rapid pulse, and an elevated sedimentation rate. Radioiodine uptake may be low even though circulating thyroid hormone is high.

Sometimes the gland is largely replaced by dense hyaline connective tissue, with extension of the fibrotic process beyond the thyroid capsule. In this invasive form of fibrotic thyroiditis (Riedel's struma the thyroid gland is woody hard and fixed. Esophageal constriction and dysphagia may be major complications of fibrotic thyroiditis.

The most common form of chronic thyroiditis is associated with enlargement of the gland and infiltration by lymphocytes. Lymphoid nodules with germinal centers are sometimes found. Often one sees a mixed process of inflammation. There are many small and large lymphocytes, plasma cells, and macrophages that are prominent especially within the colloid. The colloid appears thin and foamy. Phagocytosis of the colloid can sometimes be demonstrated by proper staining. Eosinophils may be seen with special stains Fibrosis may be evident in some portions of the gland The follicular basement membrane may appear fragmented when viewed in the electron microscope. The thyroid is usually moderately enlarged but may be small in size and weight. If inflammation is extensive. it may invade the surrounding capsular tissue.

In North America, goitrous thyroiditis is associated with HLA-DR8 in the absence of any significant association with the HLA-A or -B loci. However, in Japan. an increase in HLA-Bw35 has been reported in patients with thyroiditis.

In the fibrous variant of chronic lymphocytic thyroiditis, the gland contains prominent strands of fibrous connective tissue. In contrast to Riedel's struma, this form of fibrosis does not invade the surrounding muscle. Between the bands of fibrous tissue. the thyroid parenchyma shows epithelial degeneration and chronic inflammation with infiltration of lymphocytes and plasma cells. These cases are the ones most often associated with depressed thyroid function. Patients with the fibrotic or atrophic variant of thyroiditis have a significantly higher prevalence of HLA-DR3 when compared with control populations. An increase in Gm phenotype ag has also been found among patients with atrophic thyroiditis.

In chronic thyroiditis, thyroid epithelial cells show evidence of regeneration and proliferation, appearing as oxyphilic or eosinophilic (Askanazy or Hürthle) cells. The inflammatory process is commonly multifocal, so that some portions of the gland appear relatively normal while other areas are intensely infiltrated, with consequent degeneration of the epithelium. Often one can find neighboring sections in which there is evidence of an active process of regeneration. The follicular epithelium may be hypercellular, suggesting an effort to respond to thyroid-stimulating hormone.

Although the term Hashimoto's disease, or **struma lymphomatosa,** is often given to all the goitrous

forms of the disease, the simple designation chronic thyroiditis describes the broad spectrum of pathologic and clinical variants. In all forms of the disease, evidence of an immunologic response is found, but frequencies of antibodies may vary greatly (Table 31–3).

In the past few years, a new clinical entity of "silent," or painless, thyroiditis with transient hyperthyroidism has been described, accounting for 10–20% of all cases of hyperthyroidism. It is generally a benign disease with few prodromal symptoms, and the hyperthyroidism usually resolves spontaneously in about 2 months. On biopsy examination, the thyroid is seen to be infiltrated by lymphocytes. Most patients have high titers of antibodies to thyroglobulin or thyroid microsomes.

Treatment, Complications, & Prognosis

In most instances, the thyroid enlargement may persist if active treatment is not given. Administration of synthetic thyroxine usually relieves the distressing symptoms and reduces the size of the gland, probably by suppressing pituitary thyrotropin production. The neck pain may require treatment with analgesics such as aspirin. Corticosteroids reduce local inflammation and often produce a rapid decrease in titers of thyroid antibodies. In the cases that progress to hypothyroidism, chronic replacement therapy with thyroxine or some other thyroid preparation is necessary. Immunosuppressive drugs are also effective. Patients ill with "silent" thyroiditis and thyrotoxicosis show dramatic improvement with prednisone therapy but not with propylthiouracil or propranolol.

Surgery of the thyroid and use of radioactive iodine are not generally indicated in chronic thyroiditis. Therefore, the differentiation of thyroiditis from thyroid carcinoma and hyperthyroidism, respectively, is a matter of great practical importance. As a matter of fact, therapeutic doses of radioactive iodine may intensify thyroiditis. Patients with preexisting antibody seem to develop more autoantibody following radioiodine treatment. An elevation in antibody to the thyroid microsomes is especially prominent.

The patient with subacute thyroiditis generally recovers complete thyroid function, and any thyroid enlargement that may appear during the course of the disease generally disappears. The antibodies fall to undetectable levels when the inflammation has subsided. The hyperthyroidism that may accompany subacute thyroiditis does not require treatment.

PRIMARY HYPOTHYROIDISM (Adult Myxedema)

Insufficiency of circulating thyroid hormones leads to symptoms of hypothyroidism. The signs and symptoms depend greatly on the age at onset. Cretinism results from thyroid deficiency during fetal life and is characterized by irreversible arrest in development of the musculoskeletal and central nervous systems. There is no evidence that this disease is immunologic in origin. It occurs no more frequently in children of mothers with thyroid autoantibodies than in children of normal mothers, which means that thyroid antibody alone does not damage a normal thyroid gland.

Primary adult myxedema occurs without known cause, though it is reasonable to assume that immunologic processes play a role in the development of the disease. Patients typically have cold, dry skin; dry, coarse hair; constipation; intolerance to cold; and loss of vigor. The face is puffy and the complexion yellow as a result of carotenemia. Speech is slowed and thought processes retarded. In some cases, the heart rate is slowed and the heart is enlarged, with pericardial effusion. Deep tendon reflexes are characteristically slowed, with delayed recovery return. Firm myxedema is apparent under the skin. Laboratory findings usually include a decreased radioiodine uptake and low thyroxine (T_4) and triiodothyronine (T_3) levels. Serum cholesterol is high.

Primary hypothyroidism must be differentiated from thyroid failure due to pituitary insufficiency. The thyroid in pituitary insufficiency usually responds well to administration of thyrotropic hormone.

In severe primary hypothyroidism, the thyroid gland is usually atrophic, with fibrosis and only a few isolated islets of acinar tissue. The appearance is quite different from the fibrous variant of chronic thyroiditis.

Immunologically, hypothyroidism closely resembles chronic thyroiditis. Circulating antibodies and cell-mediated immunity to thyroid antigen are present. Antibodies are found in the same high titers characteristic of thyroiditis (Table 31–3).

HYPERTHYROIDISM

Major Immunologic Features

- Graves' disease is the most common cause.
- Sera of most patients with Graves' disease contain autoantibodies directed to the thyroid cell surface receptors for thyrotropin or thyroid-stimulating hormone (TSH).
- Some thyrotropin receptor antibodies produce hyperthyroidism by mimicking the stimulatory action of TSH, whereas others may block TSH binding.
- Autoantibodies to thyroglobulin or thyroid microsomes can be identified in many patients by a combination of hemagglutination and immunofluorescence tests.

General Considerations

In older individuals, hyperfunction of the thyroid is often insidious in onset and therefore difficult to recognize clinically. Hyperthyroidism may be due to diffuse hyperplasia (diffuse toxic goiter or Graves' disease), nodular goiter, or localized autonomous adenoma. Graves' disease (a triad of hyperthyroidism, infiltrative ophthalmopathy, and infiltrative dermopathy) is linked to thyroiditis by familial clustering; ie,

patients with thyroiditis often have family members with hyperthyroidism and vice versa. In some patients, the 2 diseases may coexist.

Because of the difficulty in recognizing mild forms of the disease, the prevalence of hyperthyroidism is not definitely known. It is clear, however, that women develop the disease 4–5 times as often as men and that the greatest incidence is in the fourth and fifth decades. In these respects also, hyperthyroidism resembles thyroiditis.

Study of the involvement of autoimmunity in Graves' disease is hampered by the lack of a well-defined experimental model. In the human disease, a relative increase of lymphocytes has been reported, with the increase in absolute numbers of peripheral B cells correlating positively with disease severity indicated by thyroid hormone levels. The thymus may be larger than normal. As mentioned previously, about one-third of patients with hyperthyroidism have autoantibody to one or another thyroid antigen. However, these antibodies might signify the simultaneous occurrence of thyroiditis and hyperthyroidism in the same patient rather than an immunologic cause of hyperthyroidism itself.

When sensitive methods are used, 50–95% of sera from patients with active Graves' disease are found to stimulate the human thyroid gland. Thyroid-stimulating antibodies (TSab) have been shown to compete with TSH for receptor sites on the thyroid cell membrane and mimic TSH activity by stimulating adenylate cyclase. It should be noted, however, that not all antibodies measured by radioreceptor assay are stimulatory. There is convincing evidence that many sera contain antibodies that compete for binding sites at the TSH receptor but lack the ability to stimulate it either in vitro or in vivo. These assays are therefore said to measure thyrotropin binding-inhibiting immunoglobulins (TBII) rather than TSAb. Volpé has recommended the general term **thyrotropin receptor antibodies (TRA)** to include these 2 opposite antibody functions. Certain TRA produce hyperthyroidism by mimicking the action of TSH, while other immunoglobulins block TSH binding and produce hypothyroidism. TRA are IgG of subclasses 1, 2, and 4 and show a wide range of isoelectric points, suggesting that the antibodies are of polyclonal origin.

Human monoclonal antibodies have been produced in the laboratory by hybridization of peripheral blood lymphocytes from Graves' patients with mouse myeloma cells. These antibodies reacted with the thyrotropin receptor, as shown by their ability to block the binding of thyrotropin. Some of these antibodies stimulated thyroid functions in the mouse bioassay and reacted with thyroid ganglioside preparations. Other monoclonals had no intrinsic stimulatory action on thyroid function, although they inhibited thyrotropin activity. These antibodies did not react with thyroid gangliosides but did bind to a glycoprotein component from human thyroid membranes.

Exophthalmos. Exophthalmos in Graves' disease is encountered in 2 forms. The staring expression commonly present in hyperthyroid patients is caused by increased sensitivity of the sympathetic innervation of the extraocular muscles to circulating catecholamines. It is alleviated by drugs such as guanethidine and specific beta blockers. This is clearly distinct from infiltrative ophthalmopathy, or endocrine exophthalmos, which is thought to be autoimmune in nature and is found in a small proportion of patients with Graves' disease (and a smaller proportion of patients with chronic lymphocytic thyroiditis). It may be very severe, progressive, and sight-threatening.

Cellular immunity to retro-orbital antigens was demonstrated in patients with exophthalmos by using leukocyte migration inhibition. Furthermore, retro-orbital muscle has affinity for both thyroglobulin and thyroglobulin-antibody complexes. Retro-orbital tissue may actually share a carbohydrate moiety of thyroglobulin. Other researchers have traced tenuous lymphatic connections between the thyroid region and the retro-orbital area. They suggested that thyroid antigens, antibodies, or immune complexes may flow from the thyroid to the orbital space and set up an immunologic reaction. Recently, circulating autoantibodies against a soluble antigen prepared from human eye muscle were detected in 17 of 23 patients with Graves' ophthalmopathy but in only 1 of 14 patients with chronic thyroiditis, in 2 of 11 patients with subacute thyroiditis, in no patient with hyperthyroidism of Graves' disease, and in none with multinodular goiter.

Treatment of infiltrative ophthalmopathy has never been satisfactory. Orbital decompression, plasmapheresis, and corticosteroid treatment have, in some cases, provided improvement. Recently, the immunosuppressive drug cyclosporine has been used to good effect in some cases. In the few patients in whom its use has been reported, immunologic improvement (restoration of normal B cell percentage and helper/suppressor T cell ratios) has been the rule, but clinical response has been variable. The value of this treatment method may depend upon the disease status—short duration, long-standing, rapidly worsening—with the impression being that progressive disease is more amenable to treatment than established disease.

Immunologic Pathogenesis

One theory of an immunologic origin of hyperthyroidism rests on the demonstration of TSab. The evidence that this factor represents a stimulating autoantibody has been outlined above. However, lack of an experimental model has hampered the study of stimulatory antibodies.

In Graves' disease, the thyroid gland is often infiltrated by lymphocytes. Both T and B cells have been identified in the focal infiltrates, with T cells predominating. Studies of the ratio of helper/inducer to suppressor/cytotoxic cell numbers made on lymphocyte populations extracted from thyroid tissue of patients with Graves' disease suggested a predominance of CD4 (helper/inducer) cells over CD8 (suppressor/cytotoxic) cells. When thyroid sections were used, however, most studies have shown that CD8 cells are

in a majority. The function and antigenic specificity of any of these cells are as yet undetermined, and their relevance to the natural history of the disease remains speculative, because preoperatively, the patients from whom thyroid tissue had been obtained had been treated with carbimazole, which has immunosuppressive properties. Even in patients with active disease, very few cells of the CD6 subset that represent antigen-presenting cells have been found, raising questions about the mechanism through which antibody production is stimulated. A possible answer is provided by the fascinating observation of the presence of HLA-DR antigens on thyroid cells from patients with Graves' disease. The normal requirement for antigen-presenting cells (macrophages) to stimulate helper T cells could be circumvented if autoantigens were aberrantly expressed on the same endocrine cell as HLA-DR antigens. Following the initial recognition of this phenomenon in thyrocytes of patients with Graves' disease by Bottazzo and his group, similar findings have been reported and confirmed in other autoimmune diseases, such as type I diabetes, alopecia areata, and primary biliary cirrhosis. Since γ-interferon can induce HLA-DR, a hypothesis for viral infections producing autoimmunity has been suggested.

Important information about the pathogenesis of Graves' disease has come from studies of HLA associations. Several studies in Caucasian populations have shown an increased frequency of the HLA-B8 antigen. The relative risk of Graves' disease in persons with HLA-B8 compared with persons lacking this antigen is about 2.5. An even closer association has been reported with HLA-Dw3, which suggests that a gene influencing susceptibility to Graves' disease is close to the D locus. HLA-Dw3 and HLA-B8 are in linkage disequilibrium.

In the Japanese, HLA-B8 is rare, and other alleles, HLA-Bw35 and HLA-Dw12, are significantly associated with Graves' disease. Among Chinese people, a significant association with HLA-Bw46 has been reported.

Clinical Features

A. Symptoms and Signs: Hyperthyroidism or Graves' disease results from overproduction of thyroid hormone. It is marked by an increased metabolic rate in most tissues of the body. It may occur in all grades of severity. Occasionally the onset is sudden, but more commonly the symptoms develop so slowly that the patient is unaware of the disease. Symptoms may be aggravated and noticeable following physical or emotional trauma. The most common symptoms are restlessness, heat intolerance, weight loss, and palpitations. The principal signs include smooth, warm, and moist skin resulting from vasodilatation and excessive sweating, a diffuse goiter, tachycardia, a wide pulse pressure, fine tremor of the hands, and proximal muscle weakness. The heart rate in hyperthyroidism is typically rapid, with an elevated systolic pressure and wide pulse pressure. In other individuals, tachycardia, arrhythmia, or evidence of congestive heart failure may first bring the patient to the physician. A striking feature of many cases of hyperthyroidism is unilateral or bilateral ophthalmopathy (also called exophthalmos) consisting of widened palpebral fissures, retro-orbital edema, proptosis, conjunctivitis, and sometimes loss of vision due to optic nerve ischemia.

B. Laboratory Findings: The histologic counterpart of Graves' hyperthyroidism is a diffusely enlarged thyroid gland with increased vascularity and varying degrees of infiltration by lymphocytes and plasma cells, often arranged to form lymphoid follicles.

Laboratory studies of patients with hyperthyroidism usually show that blood levels of thyroxine and triiodothyronine are high, and uptake of radioactive iodine by the thyroid gland is increased.

Assays of the free, non-protein-bound concentrations of thyroxine and triiodothyronine are finding wider acceptance. Immunoradiometric or fluoroimmunometric assays have greatly increased the sensitivity with which TSH concentrations can be routinely measured, and hyperthyroid value may now be clearly distinguished from those of euthyroid patients. Measurement of TSH has now become a routine test of thyroid function when either hyper- or hypothyroidism is suspected.

Immunologic Diagnosis

In most circumstances, Graves' disease can be diagnosed by clinical signs and assays for TSH, T_4, and T_3 without resorting to antibody tests. However, its insidious onset sometimes delays the recognition of hyperthyroidism. Unfortunately, not all patients with Graves' disease have TRA; as many as 50% have negative tests, depending upon the sensitivity of the assay and the clinical status. Because many patients produce both blocking and stimulating antibodies simultaneously, the balance may shift from time to time, introducing another element of uncertainty into immunologic testing.

At least 3 reliable in vitro methods for demonstrating thyroid-stimulating antibodies are now available. They measure (1) the increase in colloid droplets intracellularly, (2) increased metabolic activity of cAMP, or (3) competition with radiolabeled thyrotropin for the thyroid cell membrane. When the thyrotropin displacement assay is used, about 50–95% of patients with untreated Graves' disease are found to have significantly elevated values.

The sera of about 50% of patients with hyperthyroidism associated with either diffuse or nodular goiters contain antibodies to thyroid microsomal antigen, thyroid surface antigen, and thyroglobulin. The presence of these antibodies probably signifies inflammation and destruction of the thyroid. Elevated levels of thyroglobulin have been reported in the sera of patients with Graves' disease, with the use of a radioimmunoassay.

Differential Diagnosis

If symptoms are prominent, the diagnosis of hyper-

thyroidism usually presents no difficulty. Often, however—particularly in the early stages—the disease is mild. It may be confused with emotional anxiety states or tachycardia associated with infection or other causes. The tremor may resemble that of chronic alcoholism or parkinsonism. The diagnosis is usually based on appropriate laboratory tests. Unfortunately, the results of radioiodine uptake and serum hormone determinations may be invalidated by the use of drugs such as iodides, radiopaque contrast media, or estrogens unless the clinical situation is fairly obvious.

Treatment

The treatment of hyperthyroidism includes surgical removal of the thyroid, administration of radioactive iodine, or therapy with antithyroid drugs with adjunctive use of iodide or adrenergic blocking agents. Iodide is rarely used alone for the treatment of hyperthyroidism. It is useful in reducing the size of the gland in preparation for surgery or for promoting prompt cessation of thyroid hormone release before administration of the slower-acting antithyroid drugs. Although the reduction in size and vascularity of the thyroid gland following iodide treatment is remarkable, it is generally temporary. Antithyroid drugs such as methimazole or propylthiouracil do not prevent release of preformed hormone, so their effectiveness is slower than that of iodide. After several weeks, however, they can be quite effective in alleviating the major symptoms of hyperthyroidism. The drugs may be given continuously for control of the disease or as preparation for surgical removal of the gland.

Radioiodine (^{131}I) is widely used for the treatment of hyperthyroidism. The effectiveness of the isotope depends upon its preferential localization in the thyroid and emission of beta particles that damage the thyroid cells. The main problem in its use is to determine a dose that will alleviate symptoms without producing hypothyroidism. Part of the difficulty in determining the proper dose of radioiodine may reside in its tendency to increase sensitization. After ^{131}I treatment, patients show a transient elevation in the titer of thyroid microsomal and cytotoxic antibodies. Patients with autonomous toxic nodules rarely develop hypothyroidism or thyroid autoantibodies after radioiodine treatment. Thus, it seems that in the absence of some predisposing condition, injury to the thyroid is not sufficient to induce autosensitization.

Subtotal thyroidectomy is an effective way of treating hyperthyroidism in a properly prepared patient. Without adequate preparation, the complications of surgery are significant. In addition, removal of a hyperfunctioning gland can lead to thyroid crisis. Following surgery, it is often necessary to provide some measure of replacement with thyroid hormone. If exophthalmos is severe, treatment with anti-inflammatory corticosteroids or surgical decompression may be necessary. Radiation has also been found to be useful in treating exophthalmos.

Complications & Prognosis

Untreated hyperthyroidism can produce irreversible problems in several organ systems. There may be gradual deterioration of the cardiovascular system leading to congestive heart failure. Stressful situations, including infection, may precipitate thyrotoxic crisis, or "storm." Generally, infections are not well handled by thyrotoxic patients. Infectious hepatitis may be a serious hazard. The exophthalmos may lead to keratitis and corneal scarring or, worse, optic neuritis and blindness. Although the course of the disease may be irregular, untreated patients are always subject to recurrence and complications.

Irvine and his colleagues have shown that patients with Graves' disease who relapsed after a prolonged course of antithyroid drugs had a much higher prevalence of HLA-B8 than those who remained in remission. Similar observations have now been made with HLA-Dw3. HLA typing may eventually be useful in designing a plan of treatment in cases of hyperthyroidism.

THYROGASTRIC DISEASE & AUTOIMMUNE POLYENDOCRINOPATHY

The autoimmune response to thyroid antigens of patients with thyroiditis is strikingly analogous to the response to gastric mucosal antigens that occurs in patients with pernicious anemia. Antibodies that bind with or block the action of intrinsic factor are found in many adult patients with pernicious anemia (see Chapter 25). By immunofluorescence, antibodies to a cytoplasmic antigen of the gastric parietal cell can be demonstrated in pernicious anemia sera. These reactions are comparable to the reactions of thyroiditis patients with soluble thyroglobulin or thyroid microsomal antigen.

In addition, there is considerable overlap in the occurrence of circulating gastric and thyroid antibodies. One-fourth of patients with chronic thyroiditis were found in one study to have antibodies to the gastric parietal cell. One-third of myxedema patients were also positive. Conversely, about 30% of patients with pernicious anemia have antibody to one or another thyroid antigen in their serum. Equally interesting relationships emerge when the asymptomatic relatives of patients are studied. Forty-seven percent of relatives of patients with thyroiditis have antibody to thyroid antigens, and 18% have antibody to parietal cells. Of the relatives of patients with pernicious anemia, 45% showed antibody to the gastric parietal cells and 67% to thyroid antigens. When proper allowance is made for age and sex, patients with these organ-specific autoimmune disorders show little or no increase in the anticipated occurrence of systemic lupus erythematosus or of antinuclear antibodies.

In addition to the concurrence of autoimmunity with thyroid and gastric antigens, considerable clinical and serologic overlap has been found with other idio-

pathic endocrine deficiencies. Examples are adrenocortical insufficiency, hypoparathyroidism, pituitary failure, and the insulin-dependent form of diabetes mellitus. A higher incidence of myasthenia gravis, vitiligo, and alopecia totalis has also been reported in this group of patients. The basis of this clustering is unclear, since no single autoantigen common to these diverse cells has been discovered. However, many of these conditions, including Graves' disease, adrenocortical insufficiency, pernicious anemia, and myasthenia gravis, are associated with the HLA haplotype HLA-A1, HLA-B8, HLA-Dw3 (or DRw3).

An association of chronic mucocutaneous candidiasis with hypofunction of one or more endocrine organs has also been described, including hypoparathyroidism or adrenocortical insufficiency and other lesions in the thyrogastric group. IgA deficiency is also encountered in these disorders.

The simultaneous presence of several independent autoimmune processes and immunodeficiency suggests that these patients have some underlying defect in immunologic regulation; a deficiency in suppressor T cell capacity has been proposed. It may be that a fundamental genetic defect in suppression of autoimmune clones of lymphocytes underlies all of these abnormalities. The expression of the HLA-A1, HLA-B8, HLA-Dw3 haplotype may serve as a useful marker of this hypothetical abnormality.

CHRONIC ADRENOCORTICAL INSUFFICIENCY

Adrenal antibodies have been produced in experimental animals following injection of adrenal homogenate plus Freund's complete adjuvant. However, the reports with regard to the development of lesions have been variable in different species. Guinea pigs were found to develop lymphocytic and histiocytic infiltration and necrosis of the adrenal cortex following immunization with guinea pig adrenal extract. Cell-mediated immune reactions to adrenal microsomes were also present. Rabbits developed adrenalitis after injection of foreign but not rabbit adrenal homogenate. In inbred rats, disease could be induced by injection of syngeneic adrenal extract with Freund's complete adjuvant and pertussis vaccine. Adrenalitis was transferred from actively immunized donors to normal recipients by means of living lymph node cells. Lesions could be seen as early as 5 days after transfer. They seemed to be initiated by the arrival in the adrenal of a few specially sensitized lymphocytes that migrated by chance from the bloodstream. Their local stimulation by adrenal antigen may have caused the lymphocytes to produce mediators that attracted and activated macrophages and trapped additional nonsensitized lymphocytes at the site.

Patients with adrenocortical failure (Addison's disease) are recognized by typical symptoms of postural hypotension, weight loss, anorexia, weakness, and hyperpigmentation of folds of the skin. Acute adrenal failure sometimes leads to hypovolemic shock. It is necessary to distinguish secondary adrenal insufficiency caused by anterior pituitary failure from the primary form of the disease. In the latter instance, circulating corticotropin (ACTH) levels are usually high. Confirmatory diagnosis depends upon demonstrating low serum and urinary levels of cortisol unresponsive to ACTH stimulation. High plasma ACTH levels and low plasma and urinary cortisol levels are also indications of primary disease. Functional tests of the adrenal medulla are usually normal.

Two etiologic forms of primary Addison's disease are distinguished: exogenous and idiopathic. Tuberculous adrenal failure is now rare in countries where tuberculosis is well controlled. Systemic mycoses, metastatic tumors, irradiation, infarction, or amyloidosis may produce similar adrenocortical insufficiency. More common in Western countries is idiopathic adrenocortical failure. It occurs at any age, with a peak incidence in the fourth and fifth decades. It is seen more often in females in a ratio of approximately 2:1 or 3:1.

Histologically, in adrenocortical insufficiency, the adrenal cortex loses its normal 3-layered structure, so that the cortical cells are reduced to disorganized islets. Lymphocytic and monocytic infiltration and fibrosis are prominent. The medulla is usually normal in appearance.

There is considerable overlap of idiopathic adrenocortical insufficiency with other autoimmune diseases, including primary myxedema, chronic thyroiditis (Schmidt's syndrome), thyrotoxicosis, pernicious anemia, primary hypoparathyroidism, and diabetes mellitus. In addition to antibodies to adrenal tissue, patients with idiopathic Addison's disease frequently show antibodies to gastric parietal cells, thyroid epithelial cells, thyroglobulin, and intrinsic factor. On the other hand, the occurrence of antinuclear antibody is not unusually high in this group of patients when one takes into account their age and sex.

Antibodies to adrenal microsomes can be demonstrated by means of immunofluorescence (Table 31–1). By this method, antibodies can be found in approximately 50% of patients (38–64% in various series) with idiopathic adrenal insufficiency. The incidence of positive reactions reported in sera of patients with the tuberculous form of insufficiency varies from none to 18% in different series. Antibodies persist for many years despite replacement therapy in which adrenal hormones are used. The incidence of adrenocortical antibodies in the general population is below 5%. Normal individuals with these autoantibodies tend to have reduced adrenocortical functional reserve and sometimes progress to frank insufficiency.

In a prospective investigation of the role of adrenal autoantibodies in predicting the onset of idiopathic Addison's disease, 9 clinically normal antibody-positive subjects were followed for 42 months. In 4 of these subjects, Addison's disease developed within 1–31 months. A fifth had reduced adrenocortical reserve at the start and at the end of the investigation. An

adrenal autoantibody capable of fixing the membrane attack complex of complement was detected before the onset of the disease in the sera of the 4 patients in whom Addison's disease ultimately developed. The presence of adrenal autoantibodies may serve as a useful predictive sign of idiopathic adrenal insufficiency.

In indirect immunofluorescence microscopy, localization is usually found in the secretory cells of the zona glomerulosa, zona fasciculata, and zona reticularis. Some sera stain only one or 2 layers of the adrenal (Fig 31–3). A relatively small proportion of patients show antibodies specific for the zona fasciculata which cross-react with steroid hormone-secreting cells of the theca interna of the corpus luteum of the ovary as well as with interstitial cells of the testes and placental trophoblasts. There is said to be a correlation between the presence of these antibodies to steroid-producing cells and a clinical history of ovarian failure in addisonian patients. In young women, delayed menarche or unexplained amenorrhea has been associated with these antibodies to steroid-secreting cells. The observations support the hypothesis that ovarian disorders are related by an autoimmune pathogenetic mechanism involving antigens shared by the adrenals and ovary.

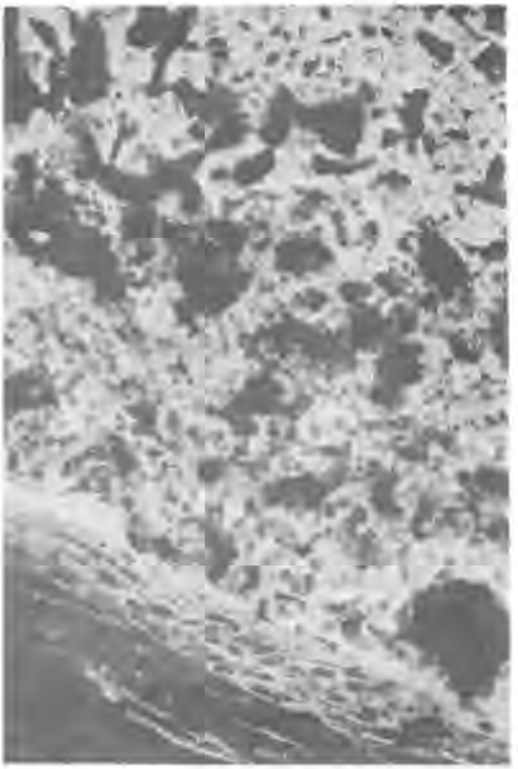

Figure 31–3. Immunofluorescent staining in adrenal insufficiency. Monkey adrenal tissue was prepared in the same manner as the thyroid tissue described in Figs 31–1 and 31–2. The tissue was not fixed and was tested with serum from a patient with adrenocortical insufficiency (diluted 1:5). Fluorescent staining of cells in the glomerulosa and fasciculata layers can be seen. (Original magnification ×180.)

PRIMARY OVARIAN FAILURE

Ovarian failure is often associated clinically with multiple features of endocrinopathy such as adrenal insufficiency, thyroiditis or thyrotoxicosis, hypoparathyroidism, and diabetes mellitus, as well as chronic candidiasis. The patients have symptoms of primary or secondary amenorrhea or premature menopause, with elevation of serum and urinary pituitary gonadotropins. A high proportion of patients with ovarian failure with associated autoimmune endocrinopathy have antibodies that are reactive with human ovarian tissue. Fluorescence is seen mainly on the cells of the theca interna, a pattern similar to that seen with antibodies to steroid-producing cells of the adrenal (Table 31–1). The antibodies can be absorbed by extracts of adrenal gland, which suggests that antigens are shared by the adrenal cortex and the gonads. Some of the sera from patients with ovarian failure are cytotoxic to luteinized human granulosa cells in the presence of complement. Antibodies are rare in patients with premature ovarian failure due to a genetic or developmental defect not associated with adrenal or other endocrine autoantibodies.

DIABETES MELLITUS

The symptomatology of diabetes mellitus is primarily a consequence of decreased production of insulin or of reduced insulin effect at peripheral target tissues, ie, insulin resistance. Decreased or absent production of insulin characterizes type I diabetes (Table 31–4), which is defined as insulin-dependent and ketosis-prone. Insulin resistance is instead a major pathogenetic contributor to type II diabetes, defined as

Table 31–4. Types of diabetes mellitus.

	Type I	Type II
Usual age at onset	< 30	> 40
Peak age	< 15	> 50
Onset	Often rapid	Insidious
Obesity	Uncommon	Common
Ketoacidosis	Common	Uncommon
Insulin dependence	Common	Uncommon
Beta cell decrease	Usually present	Usually absent
Insulitis	Common	Uncommon
Prevalence in population	< 0.5%	2%
Twin concordance	Low	High
HLA association	A1, B8, DR3 A2, B15, DR4	None known
Islet cell antibodies	Common early	Rare
Other endocrine autoantibodies	Relatively common	Rare

insulin-independent and ketosis-resistant. While in the majority of cases insulin resistance stems from a decreased number of insulin receptors on target tissues or from defective events after insulin is bound, in a small number of patients striking resistance to insulin may develop on the basis of autoantibodies competing with insulin for binding to tissue receptors (antireceptor antibodies). In most sera studied, activity resided in polyclonal molecules of the IgG class, although IgM activity was also detected. These antibodies may act in vitro as potent insulin agonists, although chronic exposure to them in vivo mostly results in an insulin-resistant diabetic condition. Occurrence of anti-insulin receptor antibodies has thus far been described as part of the syndrome that includes the skin condition acanthosis nigricans and a number of autoimmune features ranging from well-defined autoimmune diseases to combinations of elevated sedimentation rate, leukopenia, hypocomplementemia, and antinuclear antibodies. The syndrome is quite rare, has been seen mostly but not exclusively in females, and appears not to be age-related.

More intriguing—and epidemiologically more important—is the relationship between the pathogenesis of insulin deficiency in type I diabetes (juvenile-onset, insulin-dependent) and autoimmune phenomena.

Autoimmunity in Type I Diabetes

Immune phenomena in pancreatic islets. Gepts reported in 1965 that the pancreatic islets of patients with juvenile diabetes who had died soon after clinical onset of the disease showed "insulitis": peri-insular and intrainsular mononuclear cell infiltration, suggestive of a possible role for autoimmunity in attacking and destroying insulin cells. More recently, Bottazzo and colleagues have provided a detailed immunologic characterization of the insulitis. The lymphocytes infiltrating the islets of Langerhans are predominantly CD8 cytotoxic/suppressor T cells, but CD4 helper/inducer T cells and NK cells are also present. Most of these cells are "activated," since they express HLA-DR antigens and the IL-2 receptor. Preplasma cells synthesizing IgG are found at the periphery of the islets, and in most islets there is abnormal deposition of IgG and C9. These data support the participation of both humoral and cell-mediated autoimmune phenomena in the pathogenesis of type I diabetes.

A striking feature of the process leading to type I diabetes is the specificity of destruction of beta* cells (the insulin-producing cells); islet cells producing glucagon, somatostatin, and pancreatic polypeptide remain unaffected. The finding that occasional beta cells (but not other endocrine islet cells) express HLA-DR suggests that selective aberrant HLA-DR expression—similar to that observed in thyrocytes isolated from glands of patients with Graves' disease—may contribute to the targeting of the offending immune process.

*Pancreatic beta cells are now called B cells in most modern texts. In this immunology text, the authors prefer the older term beta cells to avoid any possible confusion with lymphocytic B cells.—*The Editors.*

Humoral Autoimmunity

Circulating antibodies against islet cells (ICAs) were first identified in female patients with Addison's disease, and then found in 38% of individuals with at least one other manifestation of autoimmune disease besides diabetes and in variable proportions of type I diabetics without other autoimmune manifestations. It is now known that variations in prevalence of ICA, especially in diabetics without other organ-specific autoimmune disorders, are related to the duration of diabetes; detectable in 70–85% of cases at the time of diagnosis, these antibodies tend to disappear from the serum after about a year. Persistence of ICA several years after the onset of diabetes has been noted in association with autoimmune polyendocrine diseases and, in some but not all studies, with the presence of HLA-B8 and HLA-DR3. ICAs are demonstrable with the technique of indirect immunofluorescence using fresh group O human pancreas either quick-frozen in liquid nitrogen and then used unfixed or fixed in acetone. These antibodies react against cytoplasmic antigens present in all islet cells, not just beta cells, and they are not species-specific, since positive sera react also with rat, guinea pig, and rhesus monkey islets. In human pancreatic sections, the autoantigen detected by ICA has the properties of a sialic acid-containing glycolipid. The titer of ICA is generally much lower than that of thyroid or gastric antibodies in patients with autoimmune thyroid disease or in pernicious anemia. They are exclusively IgG, mostly of subclasses IgG2 and IgG4. Approximately 70% of the ICA present at the time of diagnosis are complement-fixing, but this proportion declines to less than 50% in long-standing cases.

The prevalence of ICA in the nondiabetic adult population is 0.4%, but in relatives of individuals with type I diabetes and in school children in the USA, the prevalence has been reported to be 0.8%–1%.

Another technique used for detection of humoral autoimmunity in type I diabetes has employed dispersed rat islet cells isolated by collagenase treatment. This technique identifies antibodies against the islet cell surface (ICSAs) which are organ-specific but obviously not species-specific. In the original study by Lernmark, such antibodies (again exclusively IgG) were detected in 38% of patients at the time of diagnosis; in a subsequent study, such antibodies were detected in approximately 50% of patients with type I diabetes and in 25% of nondiabetic first-degree relatives. ICSAs are most likely different from ICAs, since the 2 types of antibodies can be detected independently of each other in diabetic sera.

Clinical and in vitro studies suggest that these antibodies may be implicated in initiating or amplifying beta cell damage. The proportion of patients with mild diabetes who become insulin-dependent in the long term is much greater among those who have circulating ICA. Prospective evaluation of siblings of type I

diabetic probands has shown that all those who eventually developed diabetes were positive for ICA up to 8 years before clinical onset of the disease. During this period, progressive blunting of the insulin response to glucose could be documented. Sera containing both ICA and ICSA have been shown to precipitate a protein having a molecular weight of 64,000 from lysates of human islet cells, and the same sera were shown to inhibit glucose-induced insulin release. Sera containing ICSA have been observed to induce complement-dependent lysis of rat islet cells and preferentially of beta cells, whereas sera positive only for ICA did not manifest these cytotoxic properties. On the other hand, the mere presence of ICSA does not appear sufficient to produce diabetes, since up to 25% of nonaffected first-degree relatives of diabetic probands have circulating ICSA. Only prospective studies of such high-risk cohorts will indicate whether the presence of ICSA consistently precedes the development of clinical diabetes.

Other classes of autoantibodies are being described in recent-onset type I diabetics—anti-insulin antibodies prior to initiation of insulin therapy, antibodies to nucleic acids, antibodies to the insulin receptor, and cold-reacting lymphocytotoxic antibodies. Whether these antibodies are related to a possible viral cause of type I diabetes or simply reflect broad activation of the immune system remains to be established.

Cellular Autoimmunity

Nerup first reported in type I diabetic patients inhibition of migration of leukocytes specific for antigens of the endocrine pancreas. Such inhibition, confirmed using purified T lymphocytes, can be reversed by addition to the test system of normal lymphocytes, suggesting that the diabetic patients have deficient suppressor T cell function. Suppressor T cell activity, demonstrated at the clinical onset of diabetes, has been shown to return to normal within 6 months after diagnosis. Increased numbers of Ia antigen-bearing T cells have been reported, both in the circulation and in pancreatic islets, indicating T cell activation analogous to that observed in other autoimmune diseases. Lymphocytes of patients with variable duration of type I diabetes but not of normal controls have been found to adhere to cultured insulinoma cells and to exert some degree of cell-mediated or antibody-dependent cytotoxicity. The best evidence for an essential role of cell-mediated autoimmune processes in the development of diabetes has been provided in experimental models. The striking "insulitis" and clinical diabetes that result in laboratory animals from the administration of multiple subdiabetogenic doses of streptozocin do not develop in athymic nude mice, whose susceptibility to the lesion and the disease are, however, restored by thymus graft. Moreover, splenic lymphocytes from euthymic littermates previously made diabetic induced transient glucose intolerance in the nude mice. The spontaneous diabetes that develops in approximately 30% of a colony of Bio Breeding/Worcester rats is phenotypically very similar to human type I diabetes—hypoinsulinemia, hyperglucagonemia, ketoacidosis, mononuclear infiltration of pancreatic islets with destruction of beta cells, and beta cell-specific ICSAs. This form of diabetes can be transferred reproducibly by injecting Con A-treated spleen cells from animals with acute diabetes into young unaffected recipients. Diabetes in the Bio Breeding/Worcester rats can be prevented by neonatal thymectomy, antiserum to rat lymphocytes, bone marrow transplantation, and cyclosporine (the immunosuppressive drug highly effective against T cell responses). Administration of silica has also been reported to prevent diabetes in this model, suggesting a critical role for macrophages in the pathogenesis of the process.

Genetics & Environment

Further support for an autoimmune contribution to human type I diabetes is provided by the association of this disease with HLA haplotypes highly prevalent in disorders of well-established autoimmune etiology. Both HLA-DR3 and HLA-DR4 show a strong positive association with insulin-dependent diabetes in Caucasians, American blacks, and Mexican-Americans. The associations with HLA-B specificities (B8, B15, and B18) are clearly secondary to the linkage disequilibrium existing between these latter specificities and DR3/DR4. At least in Caucasians, the diabetes risk conferred by DR3/DR4 heterozygosity is much higher than the simple summation of the relative risk computed for individual DR3 or DR4 positivity. Studies of families with 2 or more siblings with juvenile-onset diabetes have disclosed that affected patients share identical HLA haplotypes with significantly higher frequency than expected (55% concordance observed versus 25% expected). Recent molecular cloning of HLA-D region genes and hybridization studies have revealed that HLA-DR-identical nondiabetic subjects and type I diabetic patients differ for the presence of certain restriction fragments. Structural analysis of such diabetes-related sequences should help clarify the mechanisms underlying the association of certain HLA specificities with increased susceptibility to type I diabetes.

The individual's genetic patrimony, while it appears to be an important determinant of susceptibility to type I diabetes, does not exhaust the etiologic possibilities, since concordance for the disease both in identical twins and in siblings sharing both HLA haplotypes does not exceed 50%. Environmental factors whose diabetogenic potential is perhaps expressed or magnified through autoimmunity must then be postulated. It is likely that viruses may represent environmental contributions—and indeed, in humans, correlation between viral infections and development of juvenile-onset diabetes has been reported in cases of mumps, rubella, and infection with coxsackie B viruses and encephalomyocarditis virus. Particular strains of the latter 2 viruses have been found to have a selective tropism for pancreatic beta cells in animals and to elicit mononuclear infiltration of pancreatic

islets. A type 4 coxsackie B virus has been isolated from the pancreas of a child with recent acute onset of diabetes and found capable of causing beta cell necrosis in recipient animals. However, epidemiologic studies and the detection of ICA long before the clinical onset of symptoms militate against a close temporal relationship between coxsackievirus infection and development of diabetes in most cases. Nevertheless, a triggering effect of a virus remains appealing. In mice, experimental infection with reovirus type 1 results in a polyendocrine disease with autoantibodies to the pancreas, anterior pituitary, thymus, and gastric mucosa. This situation may be a possible model for a link between a viral infection and the autoimmune phenomena observed at the onset of type I diabetes.

Implications for Prevention & Treatment

Several attempts have been made to influence the course of type I diabetes by immunotherapy. Plasmapheresis, prednisone, and interferon have proved unsuccessful. Treatment with cyclosporine initiated within 6 weeks after diagnosis has resulted in improved insulin secretion and cessation of the need for exogenous insulin. However, interrupting cyclosporine treatment was followed by relapse, and there was significant drug-related toxicity, especially nephrotoxicity.

A powerful demonstration of immune activity associated with type I diabetes has been clearly provided by the results of pancreatic transplantation between identical twins who were discordant for type I diabetes. The recipient twins manifested a recurrence of type I diabetes, documented by appearance of insulitis in the previously normal transplanted pancreas and cessation of insulin secretion. These observations highlight the need for developing immunotherapy based on more detailed knowledge of the nature, kinetics, and duration of the events leading to type I diabetes.

IDIOPATHIC HYPOPARATHYROIDISM

Parathyroid failure is predominantly a disease of childhood and adolescence. Idiopathic hypoparathyroidism in association with adrenocortical failure is found most often in younger individuals. Histologically, idiopathic hypoparathyroidism is characterized by lymphocytic infiltration of the parathyroid glands and atrophy of glandular secretory cells. Antibodies specific for parathyroid have been demonstrated by indirect immunofluorescence in about a third of patients with idiopathic hypoparathyroidism (Table 31–1). Many of these patients also had antibodies to adrenal or thyroid tissues. Another feature of many cases of primary hypoparathyroidism is the occurrence of refractory mucocutaneous candidiasis.

Experimentally, it has been demonstrated that repeated injection of homologous parathyroid tissue into dogs may induce the characteristic biochemical changes of hypoparathyroidism. Histologic damage to the gland can be demonstrated, and parathyroid autoantibodies are found in low titer in dog serum by complement fixation.

PITUITARY FAILURE

Serologic and histologic evidence of autoimmunity to pituitary tissue has been found in a few patients. Antibodies to prolactin-secreting cells have been found most frequently in the sera of patients with multiple autoimmune endocrinopathies, especially those with idiopathic hypoparathyroidism. A second antibody has been identified that reacts with the growth hormone-producing cells of the anterior pituitary. This antibody may have diagnostic value, because several of the positive sera were taken from children with growth retardation. Lymphoplasmacytic infiltration of the hypophysis, which has recently been demonstrated by biopsy, seems to be confined to women and mostly to those who are pregnant or just postpartum. The patients presented with space-occupying lesions of the pituitary, causing visual field disturbances, but there was no histologic evidence of adenoma. In only one of 10 cases were antibodies to pituitary cells also detectable.

GUT-RELATED ENDOCRINE CELLS

The importance of the gut as an endocrine organ has only recently been fully appreciated. With the advent of immunocytochemistry in the early 1970s, it was recognized that the "clear cells" scattered among the intestinal epithelial cells are the source of several important peptide hormones. By the use of indirect immunofluorescence, antibodies have been demonstrated in patient's sera that react specifically with the cells secreting secretin, gastric inhibitory polypeptide (GIP), somatostatin, and enteroglucagon. In one study by Doniach and Bottazzo, 26 of 173 (15%) serum samples from patients with celiac disease gave positive reactions with the cytoplasm of single cells located in the epithelium of the duodenal villi or crypts of Lieberkühn. These antibodies were shown to be directed to cells containing secretin or GIP. Other investigators demonstrated antibodies to GIP-producing or somatostatin-producing cells in sera of patients with the non-insulin-dependent type of diabetes mellitus. Sera of patients with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis sometimes react with cells containing GIP, somatostatin, or enteroglucagon. These findings suggest that the diffuse endocrine system that coordinates the functions of the digestive tract may sometimes come under autoimmune attack.

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32 Neurologic Diseases

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The role of immunologic mechanisms in diseases of the nervous system has become the focus of increased interest and investigation. In diseases such as acute idiopathic polyneuropathy (Guillain-Barré syndrome) and postinfectious encephalomyelitis, the host response to an infectious agent may trigger the immune system to make a direct auto-aggressive assault on the nervous system. The role of immune mechanisms in the pathogenesis of multiple sclerosis is much less clear, but evidence from immunogenetic studies, the response to viral antigens, the oligoclonal nature of immunoglobulins found in cerebrospinal fluid, and changes that have been observed in immunoregulatory subpopulations of T lymphocytes during the course of the disease suggest that the immune response is involved in the pathogenesis of the disease. In myasthenia gravis, an auto-aggressive antibody response directed against acetylcholine receptor protein at the myoneural junction is directly involved in disease pathogenesis. In other diseases such as subacute sclerosing panencephalitis (SSPE), myotonic dystrophy, amyotrophic lateral sclerosis (ALS), and some chronic neuropathies, abnormal immune responses are known to occur; however, their pathogenetic role is unclear. In the subacute spongiform encephalopathies—a group of diseases of the nervous system in which a transmissible agent or agents different from known conventional human and animal viruses have been found—immune responses have for the most part been normal. However, strain differences in production and onset of experimental disease and the possibility that factors which affect the immune response may influence disease outcome have stimulated interest in the role of the host immune response in these conditions.

DEMYELINATING DISEASES

The demyelinating diseases of the central and peripheral nervous systems have unique pathologic, epidemiologic, and clinical features which suggest that immunologic mechanisms may play a role in the etiology and pathogenesis of some of these disorders. The commonly accepted pathologic criteria for a demyelinating disease are destruction of myelin sheaths of nerve fibers and relative sparing of axis cylinders and other elements of the nervous system. These lesions are frequently perivenous in location and are accompanied, at least in the acute phase, by an inflammatory infiltrate that is primarily of a mononuclear cell type. Two diseases of the central nervous system—acute disseminated encephalomyelitis and multiple sclerosis—and one disease of the peripheral nervous system—idiopathic polyneuritis (Guillain-Barré syndrome)—meet these criteria.

ACUTE DISSEMINATED ENCEPHALOMYELITIS

Major Immunologic Features

- Follows infectious diseases or immunization against them.
- Animal model is experimental allergic encephalomyelitis.
- Cellular but not humoral immunity to basic protein of myelin is present during the illness.

General Considerations

Although acute disseminated encephalomyelitis is an uncommon disease of the nervous system, it has become important because of the widespread practice of vaccination for prevention of infectious diseases throughout the world. The incidence is not related to race, age, or sex. The onset of clinical illness can be several days to weeks following vaccination, or, in the case of natural infection with viruses such as measles, rubella, varicella, mumps, and influenza, it can occur concomitantly with the illness (parainfectious) or following the acute phase of the illness (postinfectious). The lesions of acute disseminated encephalomyelitis are marked by perivascular mononuclear cell infiltrates in white matter throughout the brain; polymorphonuclear leukocytes are usually seen in the acute hemorrhagic form of the disease. As the lesions age, they tend to become less inflamed and more sclerotic, with proliferation of astrocytes and formation of gliotic scars in the center. The lesions of acute disseminated encephalomyelitis are pathologically all of the same age, which reflects the monophasic clinical character of the illness.

Immunologic Pathogenesis

The major arguments favoring an immunologic explanation of this disorder are that multiple infectious

agents or vaccines produce a single stereotyped lesion in the central nervous system and that this same lesion can be produced in several animal species by inoculation of brain material. Experimental allergic encephalomyelitis (EAE) is an autoimmune disease in which an animal is immunized with homologous or heterologous extracts of whole brain, the basic protein of myelin, or certain polypeptide fragments of basic protein together with Freund's complete adjuvant. Ten to 21 days following immunization, an illness ensues characterized by lethargy, weight loss, tremor, hind limb paresis, loss of sphincter control, and (frequently) death. The lesions are all of the same age and consist of perivascular mononuclear cell infiltrates in white matter with varying degrees of demyelination. After immunization, cellular immunity to myelin basic protein can be demonstrated by skin testing as well as by in vitro tests such as inhibition of leukocyte and macrophage migration and lymphocyte activation (see Chapter 18). Antibodies to basic protein and to myelin appear but do not correlate positively with disease production. EAE can be transferred by sensitized lymphocytes but not by serum.

Cellular immunity to basic protein has been demonstrated in acute disseminated encephalomyelitis by measuring the activation of peripheral blood or spinal fluid lymphocytes (see Chapter 18). The response tends to be highest during the acute phase of the illness and decreases as the disease progresses. No antibodies to basic protein of myelin have been demonstrated. Cerebrospinal fluid may be abnormal, with mild to moderate pleocytosis and mildly elevated total protein. The proportion of γ-globulin in the cerebrospinal fluid may also be increased, but there is no evidence that this represents local immunoglobulin production in the central nervous system as occurs in multiple sclerosis.

Clinical Features

Systemic symptoms such as fever, malaise, headache, myalgia, nausea, and vomiting generally precede neurologic symptoms by 24–48 hours. Neurologic symptoms develop rapidly thereafter and include pain, numbness, paresthesias, motor weakness, incoordination, and bulbar symptoms such as dysarthria, dysphagia, pooling of pharyngeal secretions, and respiratory distress. Spasticity, extrapyramidal signs, and pathologic reflexes are commonly seen. Visual defects can occur if the optic nerve is involved, and widespread lesions in the brain can lead to stupor and coma. Seizures are common in severe cases and in the acute hemorrhagic form.

Differential Diagnosis

Acute disseminated encephalomyelitis must be differentiated from other diseases that can acutely and diffusely affect the central nervous system. Acute multiple sclerosis without a history of neurologic dysfunction can be difficult to differentiate from acute disseminated encephalomyelitis. The presence of fever and a preceding viral illness or vaccination favors the latter diagnosis. Autoimmune vasculitis such as occurs in systemic lupus erythematosus (SLE) can affect the central nervous system primarily, although neurologic symptoms generally tend to occur during the course of the systemic illness and tend to be more focal than in acute disseminated encephalomyelitis. Evidence of vasculitis in other organs is helpful in distinguishing these diseases from acute disseminated encephalomyelitis. Primary infections of the nervous system with herpes simplex, measles, mumps, and rubella viruses as well as with the arboviruses tend to involve gray matter as well as white and produce more evidence of neuronal dysfunction such as seizures, stupor, coma, and extrapyramidal signs early in the illness. Direct isolation of viruses from cerebrospinal fluid as well as a rise in serum antibody titer is helpful in differentiating viral encephalitides from acute disseminated encephalomyelitis. Toxoplasmosis, which can directly infect the central nervous system and presents as an acute disseminated encephalitis, can be diagnosed serologically. However, failure to find evidence for an infectious cause may necessitate a diagnostic brain biopsy.

Treatment

Although the course of acute disseminated encephalomyelitis is unpredictable, corticosteroids may be of value in treatment. The successful suppression of experimental allergic encephalomyelitis and clinical recovery of animals after receiving injections of basic protein, encephalitogenic peptides, or monoclonal antibodies to T lymphocytes offer hope that by manipulating the immune system a state of immune paralysis or tolerance can be produced, resulting in cessation of the attack of sensitized lymphocytes on the nervous system.

Complications & Prognosis

The mortality rate from acute disseminated encephalomyelitis varies from 1% to 27%, with a higher rate reported in cases associated with measles. Major and minor neurologic sequelae persist in 25–40% of survivors. The occasional occurrence of relapses blurs the distinction between this condition and multiple sclerosis in about 5% of cases.

MULTIPLE SCLEROSIS

Major Immunologic Features

- Inflammatory demyelination in central nervous system white matter.
- Increased level of cerebrospinal fluid IgG containing oligoclonal bands.
- Increased levels of antiviral antibodies in serum and cerebrospinal fluid.
- Alterations in immunoregulatory T cells during the course of the disease.

General Considerations

Multiple sclerosis is a chronic relapsing disease in

which there are signs and symptoms of multiple areas of central nervous system involvement both in time and in space. It is one of the most prevalent diseases of the central nervous system, and, because young adults are frequently affected with debilitating symptoms lasting many years, it represents a serious public health problem. Epidemiologic studies have uncovered important clues about multiple sclerosis, but the cause remains unknown. Low-risk areas of the world have a prevalence of 5–10 cases per 100,000 population; high-risk areas have a prevalence of 50–100 per 100,000 population. Individuals who migrate from high-risk to low-risk areas, or vice versa, carry their native risk of acquiring the disease with them if they move after age 15. In addition, there is a peak onset at age 30, with few cases before age 15 or after age 55, suggesting that some critical event in determining the risk of acquiring multiple sclerosis occurs in adolescence. Familial cases are not uncommon, and the risk of a first-degree living relative having the disease is 10–15 times higher than the risk in the rest of the population. The disease is rare among Orientals and Africans. A consistent finding in several large studies of multiple sclerosis patients has been slightly but persistently elevated serum and cerebrospinal fluid antibody titers to measles and, less commonly, to other viruses such as vaccinia, mumps, rubella, and herpesviruses. These findings suggest that a latent viral infection may play a role in the etiology of multiple sclerosis. This argument is supported by epidemiologic studies in the Faroe Islands, where the temporal clustering of cases suggests a transmissible cause, possibly associated with an epidemic of canine distemper. Several studies of histocompatibility antigens in multiple sclerosis patients have indicated a statistically significant but low-level association with HLA-A3 and HLA-B7 in Northern European and North American populations. A higher level association has been demonstrated with HLA-DR2, which is linked to HLA-A3 and HLA-B7 and may be more closely linked to a multiple sclerosis-susceptibility gene.

Immunologic Pathogenesis

The lesions of multiple sclerosis are confined to the central nervous system and involve primarily the white matter in the periventricular areas of the cerebrum, cerebellum, brain stem, and spinal cord. In early active disease the lesions consist of inflammatory demyelination with mononuclear infiltrates; older lesions consist of plasma cells, mature lymphocytes, macrophages, and astrocytes. Unlike the lesions of acute disseminated encephalomyelitis, these lesions appear to be of different ages and correlate with the appearance of clinical signs and symptoms at different times during the illness. The lesions of multiple sclerosis, termed plaques, contain plasma cells and immunoglobulin. This finding is reflected in the cerebrospinal fluid, where increased concentration of γ-globulin can be found in 60–80% of multiple sclerosis patients. The ratio of cerebrospinal fluid to serum immunoglobulin is increased and the cerebrospinal fluid IgG index is elevated in over 85% of patients with active multiple sclerosis.

$$\frac{\text{CSF IgG} \div \text{Serum IgG}}{\text{CSF albumin} \div \text{Serum albumin}} = \text{CSF IgG index}$$

This index is also elevated in other inflammatory nervous system disease and indicates that local IgG synthesis is occurring within the nervous system. The immunoglobulin has an oligoclonal pattern on electrophoresis, which is further evidence for local central nervous system production. A small portion of the oligoclonal immunoglobulin has been shown to react with measles and other viral antigens and myelin basic protein; the reactivity of the remaining immunoglobulin is still unknown.

The similarities between the lesions of multiple sclerosis and the inflammatory demyelination seen in experimental allergic encephalomyelitis (particularly in the relapsing form) have suggested that immune mechanisms may be involved in the pathogenesis of multiple sclerosis. In experimental allergic encephalomyelitis, disease induction requires that a species-specific encephalitogenic determinant be presented to the immune system.

In multiple sclerosis, the mechanism by which such an event might occur is unclear; however, viral infection is a likely possibility. Inflammatory demyelination has been described in animals infected with both DNA- and RNA-containing viruses. In some cases, demyelination occurs as a direct result of viral infection of oligodendrocytes, the myelin-synthesizing cells; in others, the infection may induce sensitization to antigenic determinants of myelin, resulting in a cell-mediated autoimmune response. Amino acid sequences present in myelin basic protein and common to certain viruses have been identified that could be responsible for such cross-reactivity by a process of molecular mimicry. Insertion of viral proteins into glial membranes and incorporation of antigenic determinants of myelin into viruses are other mechanisms by which an autoreactive process could arise. An additional role for viral infection in multiple sclerosis is suggested by the finding that acute exacerbations are often preceded by mild respiratory or other viral illnesses, raising the possibility that the infection may act as a trigger to activate a primed immune system. Recent evidence that viral infection may induce class II histocompatibility antigens on monocytes and astrocytes, probably via production of gamma interferon, suggests a mechanism by which such immune activation could occur.

There is abundant evidence of abnormal T cell-mediated immunoregulation in multiple sclerosis. It is generally accepted that suppressor T cell function is deficient during disease activity, as shown by a relative loss of ability to generate suppression by incubation of peripheral blood mononuclear cells with Con A. Studies of T cell subsets with monoclonal antibodies have generally led to conflicting results with respect to suppressor T cells during exacerbations; how-

ever, abnormally activated T cell subsets have been identified in peripheral blood and cerebrospinal fluid using monoclonal antibodies. The increased levels of IgG in the cerebrospinal fluid of multiple sclerosis patients point to a defect in regulation of immunoglobulin production by B lymphocytes and plasma cells. Abnormalities of natural killer (NK) cell activity and interferon production have also been described, although the origin of these defects and their roles in the pathogenesis of multiple sclerosis remain uncertain. Helper T and suppressor T cells and macrophages have been identified in active plaques that participate actively in myelin breakdown as well as in their more pedestrian function as scavengers of damaged nervous tissue. The relationship between these defects of immunoregulation, the genetics of multiple sclerosis, the possible role of viral infection, and the effector mechanisms that result in demyelination are exceedingly complex and remain highly speculative.

Clinical Features

Since the lesions of multiple sclerosis tend to involve many areas of the central nervous system white matter, the symptoms and signs of the disease are extremely varied. The most common manifestations are the development of motor weakness, paresthesias, impairment of visual acuity, and diplopia. These symptoms may occur rapidly, as acute exacerbations that develop over several days and persist for days to weeks with gradual recovery—or more slowly in the chronic progressive form of the disease. Subsequent exacerbations occur at widely varying intervals and tend to subside with less complete recovery of function and more chronic disability as the disease progresses. Ataxia of gait, urinary bladder dysfunction, impotence, spasticity, and, in late stages of the disease, mild to moderate dementia are common.

Differential Diagnosis

Acute episodes of multiple sclerosis must be differentiated from other structural lesions of the central nervous system. Spinal cord compression due to tumors or spondylosis usually presents with evidence of partial or complete intraspinal subarachnoid block, and the cerebrospinal fluid protein is elevated to much higher levels than in multiple sclerosis. Central nervous system tumors, both primary and metastatic, can mimic multiple sclerosis and must be ruled out with appropriate neurodiagnostic procedures. The symptoms of neurosyphilis may mimic multiple sclerosis, but a history of syphilitic infection or the presence of a positive serologic test for syphilis is helpful in establishing the diagnosis. Vasculitides such as SLE have been mentioned previously in the discussion of acute disseminated encephalomyelitis. Finally, degenerative spinal and cerebellar disorders such as Friedreich's ataxia, parenchymatous cerebellar degeneration, and olivopontocerebellar degeneration can all mimic multiple sclerosis. These disorders tend to be familial, chronically progressive, and not associated with the elevation of spinal fluid γ-globulin that occurs in the majority of cases of multiple sclerosis. Procedures helpful in establishing the diagnosis are measurement of the IgG index and levels of myelin basic protein in cerebrospinal fluid, electrophoresis of cerebrospinal fluid to detect oligoclonal bands, visual and auditory evoked potentials, and CT scanning. Magnetic resonance imaging has proved to be highly sensitive for the visualization of plaques and may become the diagnostic technique of choice.

Treatment

While experimental allergic encephalomyelitis can be successfully suppressed with myelin basic protein and related antigens as well as with immunosuppressive agents such as corticosteroids and cytotoxic drugs, patients with multiple sclerosis have shown less clear benefit from such treatment. Corticosteroids or corticotropin may shorten the duration of acute exacerbations, particularly when optic neuritis is present, but the long-term benefits of such therapy are not striking.

Numerous trials of experimental immunotherapy have been undertaken, some of which have met with partial success. Intensive immunosuppression with cyclophosphamide combined with corticotropin seems to arrest progression in some patients for periods up to 1 year. Trials of alpha interferon given by subcutaneous injection and beta interferon given intrathecally have been reported to prevent exacerbations in patients with relapsing-remitting multiple sclerosis. A synthetic polypeptide that inhibits experimental allergic encephalomyelitis in animals has been effective in preventing exacerbations of multiple sclerosis in preliminary trials; its effectiveness in large-scale controlled studies remains to be determined. Although there is little evidence for a pathogenic humoral factor in multiple sclerosis, plasmapheresis has been reported to produce improvement and deserves to be studied further. A multicenter study of cyclosporine in patients with chronic progressive disease is currently under way and represents an attempt to affect the clinical course by selectively inhibiting helper T function while sparing suppressor T cells. Targeted immunotherapy using monoclonal antibodies directed against subpopulations of activated T cells is also in the preliminary stages of investigation. It remains to be seen which, if any, of these therapeutic modalities will survive testing in rigorously controlled double-blind clinical trials.

Complications & Prognosis

The prognosis of multiple sclerosis is difficult to predict because of the extremely variable nature of the disease. Benign cases in which patients have functioned normally or with little deficit after initial episodes are not uncommon. At the other extreme, fulminant cases of acute multiple sclerosis have resulted in death during the initial episode. Most patients fall between these extremes and continue to have exacerbations and remissions for many years. The average duration of life after onset of symptoms is at least 25

years. Despite substantial evidence for defective immunoregulation, patients with multiple sclerosis do not have increased susceptibility to other autoimmune disorders, infections, or neoplasms.

ACUTE IDIOPATHIC POLYNEURITIS (Guillain-Barré Syndrome)

Major Immunologic Features

- Commonly follows viral infections.
- Inflammatory demyelination of peripheral nerves.
- Pathologic similarity to experimental allergic neuritis in animals.
- Cellular and humoral immunity to nerve proteins present.

General Considerations

Acute idiopathic polyneuritis, like acute disseminated encephalomyelitis, frequently occurs following an infectious illness. Upper respiratory infections, exanthems, vaccinations, and specific viral illnesses such as measles, infectious mononucleosis, and hepatitis commonly precede acute idiopathic polyneuritis by 1–3 weeks. Approximately 1000 cases were associated with the "swine flu" vaccination program in 1976. The disease affects all age groups, and incidence is not related to sex or race.

Immunologic Pathogenesis

Acute idiopathic polyneuritis is a demyelinating disease of the peripheral nervous system characterized by a perivascular mononuclear cell infiltrate with segmental demyelination of peripheral nerves in the areas of inflammation. The inflammatory response is present even in the earliest stages. In areas of most severe involvement there is some axonal destruction and wallerian degeneration. These areas sometimes show infiltration of polymorphonuclear leukocytes as well as mononuclear cells early in the course of the disease. Later, the lesions frequently have plasma cells in the infiltrate. Experimentally, an identical clinical and pathologic entity can be produced in many animal species by the injection of peripheral nerve myelin, the P_2 protein of myelin, or peptides of P_2 protein together with Freund's complete adjuvant. This illness, experimental allergic neuritis (EAN), begins as weakness and later leads to paralysis 10–24 days after immunization. The immunopathologic nature of the illness can be demonstrated by the fact that lymphocytes from animals with experimental allergic neuritis are sensitive to extracts of peripheral nerve and purified proteins of peripheral nerve myelin and undergo activation or produce lymphokines in the presence of these antigens. Sensitivity to the immunizing antigen parallels the course of the illness. The disease can be passively transferred with sensitized cells but not with serum. Lymphocytes from animals with experimental allergic neuritis have the capacity to produce demyelination in tissue culture. Serum from animals with EAN induced by peripheral nerve myelin but not the P_2 protein will demyelinate tissue cultures. Antibody to galactocerebroside can produce a demyelinating neuropathy when injected locally into peripheral nerve or when raised in rabbits by repeated injections of galactocerebroside in Freund's complete adjuvant.

Immunologic abnormalities have also been described in acute idiopathic polyneuritis. An increased number of spontaneously transformed circulating lymphocytes has been described, as well as lymphocytes that show sensitivity to extracts of peripheral nerve and peripheral nerve myelin proteins by proliferation or lymphokine production. This sensitivity appears to be specific, since there is no sensitivity to central nervous system antigens in these patients.

Antinerve and antimyelin antibodies have been described in acute idiopathic polyneuritis; however, it has not been established whether these are pathogenic or simply reflect secondary immune reactions to nerve tissue destruction. Intraneural injection of serum from patients with acute idiopathic polyneuritis causes demyelination and nerve conduction block in rats; however, the serum factor responsible does not appear to be an antibody specific for P_2 protein or galactocerebroside.

In patients tested early in the course of their illness, high titers of complement-fixing antibody, probably IgM, have been detected. Such antibodies are also found in patients with chronic demyelinating neuropathies. Controlled clinical trials of plasma exchange have shown a beneficial effect, particularly in patients treated during the first 2 weeks of illness, tending to support the clinical relevance of a humoral factor present at the onset of the disease.

Clinical Features

The onset of acute idiopathic polyneuritis is generally characterized by a rapidly progressing weakness first of the lower extremities, then the upper extremities, and then the respiratory musculature over a period of 3–7 days. Weakness and paralysis are frequently preceded by paresthesias and numbness of the limbs, but objective sensory loss is generally mild and transient. Cranial nerves, most commonly the facial nerve, can be involved. The tendon reflexes are decreased or lost early in the course of the illness, and nerve conduction velocities in affected limbs are moderately to markedly slowed, consistent with a demyelinating peripheral neuropathy. Cerebrospinal fluid protein is increased in all cases, but frequently not during the first few days of the illness. Cerebrospinal fluid white cell counts are commonly normal or slightly elevated. The most common clinical course is one of rapid evolution of symptoms over a period of 1–3 weeks with improvement thereafter and return to normal function over a period of 6–9 months. However, other patterns such as a more gradual onset, a more prolonged period of complete paralysis, recovery with severe residual deficits, and a relapsing course have also been described.

Differential Diagnosis

Acute idiopathic polyneuritis can be differentiated from porphyric polyneuropathy by the demonstration of porphyrins in the urine of such patients. Heavy metal intoxication with lead, thallium, and arsenic can all be ruled out by appropriate blood or urine tests. Acute transverse myelitis in the early stages may resemble acute idiopathic polyneuritis, but pathologically increased reflexes and spasticity occur several days to weeks after initial flaccidity, and bowel and urinary bladder involvement are more common in transverse myelitis. Vasculitides such as polyarteritis nodosa can produce peripheral neuropathies, but these tend to present as asymmetric involvement of one or more nerves. The weakness of skeletal muscles involved in myasthenia gravis may resemble acute idiopathic polyneuritis, but the former is more likely to be associated with ocular muscle involvement and will generally respond to anticholinesterase drugs.

Treatment

The use of anti-inflammatory agents in treating acute idiopathic polyneuritis is controversial. Some patients may benefit from corticosteroids, but at least one well-controlled study has shown that these drugs tend to prolong the duration of illness and are therefore contraindicated except in some cases of recurrent polyneuropathy. Plasmapheresis seems to be effective, especially if it is begun as early as possible in the patient's illness and if concurrent steroid treatment is avoided. Intensive supportive care, including respiratory assistance, must be given as required.

Complications & Prognosis

The widespread availability of modern methods for assisting and maintaining respiration has resulted in a marked decrease in the number of fatalities from acute idiopathic polyneuritis. However, the incidence of residual neurologic deficits is higher than previously recognized and may occur in as many as 50% of cases. Persistent deficits such as weakness and loss of reflexes are understandable, since irreversible axonal disruption and wallerian degeneration occur in severely affected nerves. The mechanical problems of clearing respiratory secretions in patients with respiratory muscle and pharyngeal weakness favor the development of respiratory infections, which are a severe threat to life in hospitalized patients. Associated autonomic neuropathy may produce vasomotor instability and cardiac arrhythmias and may be responsible for unexplained sudden death despite adequate respiratory care.

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MYASTHENIA GRAVIS

Major Immunologic Features

- Commonly associated with thymoma or thymic hyperplasia.
- Pathogenic autoantibodies directed against acetylcholine receptor protein present in serum.
- Other autoantibodies are common.
- Often associated with other autoimmune diseases.

General Considerations

Myasthenia gravis is a disease of unknown cause in which there is motor weakness due to a disorder of neuromuscular transmission. The disease tends to affect young adults, more commonly women, but onset in childhood as well as onset beyond age 40 is not uncommon. Familial cases are known, but there is no racial predilection. The occurrence of myasthenia gravis with thymomas, thymic hyperplasia, autoantibodies, and certain autoimmune diseases strongly suggests that the immune system is involved in the pathogenesis of this disorder. Recent studies have shown that anti-acetylcholine receptor antibody, which binds at the myoneural junction, interrupts neuromuscular transmission by increasing endocytosis of acetylcholine receptors, and forms immune complexes that bind complement and cause additional destruction of the myoneural junction.

Immunologic Pathogenesis

Immunologic abnormalities are common in myasthenia gravis. Antibodies to striated muscle that cross-react with thymic epithelial cells have been described, as have antinuclear and antithyroid antibodies. Cellular immunity to crude muscle antigen and purified muscle protein, measured by inhibition of leukocyte migration, has been demonstrated. These abnormalities, as well as the association of myasthenia gravis with systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and thyroiditis (more commonly than would be expected by chance alone), have focused attention on the immune system. Abnormalities are present in the thymus in 80% of myasthenics, either as true thymomas (10%) or in the form of thymic hyperplasia with increased numbers of germinal centers (70%) that contain increased numbers of B lymphocytes. The fact that two-thirds of all myasthenics who undergo thymectomy show complete or partial remission also suggests that the thymus may be the site of production of a neuromuscular blocking agent.

The immunoglobulin nature of the blocking agent and its further characterization as anti-acetylcholine receptor antibody have greatly elucidated the pathogenesis of myasthenia gravis. The abnormalities in neuromuscular transmission, the immunopathologic features, and the response to treatment in clinical practice are quite similar to what is seen in experimental autoimmune myasthenia gravis produced in rabbits and rats by the injection of heterologous and homologous acetylcholine receptor protein. In both diseases, anti-acetylcholine receptor antibody is present at the myoneural junction, in the peripheral blood, and in the thymus, where it may be directed against acetylcholine receptors present on thymic epithelial cells. The antibody binds to acetylcholine receptors, which

then become endocytosed, depleting the membrane of receptor. Immunoglobulin and complement can be identified at the myoneural junction in myasthenia gravis and in chronic experimental autoimmune myasthenia gravis. Phagocytes can be seen in the acute stages of the latter but not in myasthenia gravis. The consequences of the autoimmune attack in severe and advanced cases of myasthenia gravis are that the remaining receptor is complexed with antibody and there is a simplification and unfolding of the myoneural junction. The loss of acetylcholine receptors is responsible for the decrease in the amplitude of the miniature end plate potential in myasthenia gravis. Cellular and humoral immunity to acetylcholine receptor protein occurs in both diseases. However, it appears that the antibody response is most closely associated with disease symptoms. Serum from patients with myasthenia gravis and from animals with experimental autoimmune myasthenia gravis will produce disease in recipient animals, and—while cells from experimental allergic myasthenia gravis animals will produce disease in recipients—the time course of disease production suggests that the cells responsible are antibody-producing cells.

Clinical Features

In myasthenia gravis, the muscles may be weak or normal at rest but become increasingly weaker with repetitive use. The weakness is often first noted in the extraocular muscles and manifested as diplopia or ptosis. Pharyngeal and facial muscle weakness, resulting in dysphagia, dysarthria, and difficulty in chewing, commonly occurs. Skeletal muscle weakness is more often proximal than distal, and difficulty in climbing stairs, rising from chairs, combing the hair, or even holding up the head results. All of these symptoms show fluctuations in intensity and are more severe late in the day. The neurologic examination is normal except for the muscle weakness. The disease is said to remit spontaneously in 25% of cases within the first 2 years. Serious exacerbations of the illness, including respiratory impairment—especially in elderly patients who have other complicating diseases—account for the majority of the deaths from myasthenia gravis. Modern methods of assisted respiration and constant monitoring of cardiac and respiratory function have greatly reduced the mortality rate in myasthenia gravis, which previously was about 20–30%.

The abnormality in neuromuscular transmission in myasthenia gravis, which includes a decrease in the amplitude of the miniature end-plate potential, can usually be overcome by anticholinesterase drugs such as edrophonium (Tensilon) and neostigmine. The improvement in myasthenic weakness after injection of these drugs is helpful in diagnosis.

Immunologic Diagnosis

Anti-acetylcholine receptor antibodies can be found in 90% of myasthenia gravis patients and occasionally in thymoma patients without muscle weakness. Their presence in patients with symptoms suggestive of myasthenia gravis can be considered diagnostic.

Differential Diagnosis

Myasthenia gravis can usually be differentiated from other myopathies on the basis of its response to anticholinesterase drugs. The various forms of periodic paralysis do not show the ocular muscle involvement seen in myasthenia. The myasthenic syndrome (Eaton-Lambert syndrome) usually seen in association with small cell carcinoma of the lung can be differentiated by electrodiagnostic studies as well as by its response to guanidine and lack of response to anticholinesterase agents.

Treatment

Anticholinesterase drugs such as pyridostigmine and neostigmine in combination with atropine are the most commonly used form of long-term therapy. Beneficial effects from thymectomy are seen in the majority of cases, and all patients with myasthenia gravis except those with nondisabling ocular myasthenia should be considered for thymectomy. The response to corticosteroids and immunosuppressive agents such as cyclophosphamide and azathioprine has been encouraging. These agents frequently can bring about remission and long-term control of symptoms in patients who have not responded to anticholinergic medication or thymectomy. Dramatic effects have been described in severely ill patients who have been treated with plasmapheresis. The beneficial effect of both of these therapies mayconsist of removal of large quantities of anti-acetylcholine receptor antibody. Plasmapheresis has been found to be more effective if patients are simultaneously given immunosuppressive drugs to diminish the rapid increase in anti-acetylcholine receptor antibody that follows plasma exchange.

The costs and technical difficulties involved in plasmapheresis have limited its use to severely ill patients who have failed to respond to other treatments. Remission or improvement can be induced with such treatment, which can be maintained using anticholinesterase and immunosuppressive drug therapy. Despite the complexity of plasmapheresis, its morbidity rate is quite low.

Complications & Prognosis

The course of myasthenia gravis prior to the widespread practice of thymectomy and the use of corticosteroids was one of remission in 25% of cases during the first 2 years and a course of chronic, persistent weakness with a 20–30% mortality rate in the remainder. Improvement and remission within 5 years can now be anticipated in up to 90% of patients undergoing thymectomy, and further improvement with reduction of the dosage of anticholinesterase drugs can be expected with the use of corticosteroids. Cholinergic crisis with weakness resulting from overdosage of anticholinesterase drugs, pulmonary infections resulting from pooling of pharyngeal secretions, and lowered

resistance to invading organisms resulting from corticosteroid therapy are persistent hazards for the myasthenic patient.

IMMUNOLOGIC ABNORMALITIES IN OTHER NEUROLOGIC DISEASES

In addition to the decreased levels of IgA noted in ataxia-telangiectasia (Chapter 20), abnormalities of the major immunoglobulin classes and abnormal immune responses have been described in several seemingly unrelated neurologic diseases. The relationships of the immune abnormalities and the neurologic deficits are still unclear, but there is little evidence for a causal relationship in most of these conditions.

ALZHEIMER'S DISEASE

Alzheimer's disease (presenile dementia) and senile dementia of the Alzheimer type (SDAT) are dementing diseases of unknown cause characterized by progressive memory loss, intellectual deterioration, abnormalities of personality, and language dysfunction. The onset can occur in late adult life (presenile type) or after age 65 (senile type), but in either case, the course is inexorably progressive over 5–10 years. Patients with SDAT have 5 times the mortality rate of nondemented cohorts. Death rates are higher for all causes, including infection, neoplasia, and vascular disease. The increased susceptibility to complicating illnesses is multifactorial: poor nutrition and hygiene resulting from neglect and apathy and diminished immune function all play a role.

The major pathologic feature in SDAT involves the frontal, parietal, and temporal cortices, which become markedly atrophic. Neurofibrillary tangles in neurons, senile amyloid plaques, and granulovacuolar degeneration occur in these areas. Abnormalities in neurofilament processing and function have been suggested as a common pathway through which exogenous environmental factors such as viruses, toxins, or genetic factors affecting protein or nucleic acid metabolism could act to produce this disease.

Cellular and humoral immunity are frequently abnormal in SDAT. However, the abnormalities are not disease-specific and may be the result of increased age, poor nutrition, and the debility associated with SDAT. Autoantibodies directed against cellular and subcellular components of brain probably represent a response to rather than a cause of the degenerative process. Amyloid plaques in brain led some workers to suggest that immunoglobulin might be involved. However, recent studies have shown that the amyloid is derived from degenerating neurofilament proteins and that specific serum antibodies are not involved.

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a degenerative disease of anterior horn cells (motor neurons) and the motor system, resulting in weakness, muscle wasting, hyperreflexia, and progressive debilitation leading to death in 3–5 years. The cause is unknown, but a viral infection or virus-immune mechanism has been suspected. Immunologic and immunogenetic studies on small numbers of patients have produced inconsistent results. Occasional defects in cellular immunity, increased levels of circulating immune complexes containing unidentified antigens, and altered levels of serum immunoglobulins have been reported. Some studies have shown an association of ALS with specific HLA phenotypes, but a consistent pattern has not emerged. On Guam, where a focus of ALS has been studied for many years, moderate immunodeficiency including skin test anergy, low numbers of total lymphocytes (particularly T cells), and poor functional responses of lymphocytes tested in vitro occurred in many ALS patients. The most severe immunodeficiency was associated with HLA-Bw35 and shortened survival. Serum immunoglobulin levels (IgA and IgG) were elevated and immune complexes were present in some, but a specific antiviral or autoimmune response associated with the altered serum immunoglobulin levels, the deficient cellular immune response, or differences in survival could not be identified. Therefore, it appears that neither immunodeficiency nor a specific antiviral immune response is a major feature of the disease. An autoimmune component has been suggested by studies demonstrating antibody in the serum of ALS patients capable of inhibiting "sprouting," a motor neuron response to injury in vitro. Whether this finding is a result of the degenerative process or plays a significant causal role has yet to be determined.

CHRONIC DEMYELINATING POLYNEUROPATHIES

Chronic demyelinating polyneuropathies are relatively uncommon disorders that resemble acute idiopathic polyneuritis pathologically and electrophysiologically but follow a much more indolent (frequently relapsing) course. Nerve conduction studies show profound slowing with conduction block; cerebrospinal fluid protein is characteristically increased; deposits of immunoglobulin may be found in peripheral nerve; and complement-activating antibody to myelin has been described, suggesting an immune-mediated process. In support of this hypothesis, patients frequently respond to plasma exchange, long-term corticosteroid treatment (particularly in the relapsing type), or other immunosuppressive agents.

Patients with multiple myeloma, Waldenström's macroglobulinemia, and primary systemic amyloidosis sometimes develop peripheral neuropathies in

which the histologic pattern is primarily axonal with secondary demyelination. The pathogenesis of these conditions has been largely unexplored. However, a group of individuals with benign monoclonal gammopathy and peripheral neuropathy has recently aroused a great deal of interest because of evidence that their circulating paraproteins, usually of the IgM-κ isotype, are monoclonal antibodies directed against the myelin-associated glycoprotein (MAG) of peripheral nerve myelin. These autoantibodies appear to react specifically with the carbohydrate portion of the MAG molecule and cross-react with other glycoproteins and with a glycolipid component of peripheral nerve. Therefore, the specific antigen involved in the pathogenesis of the neuropathy is uncertain. In addition, evidence that these antibodies are actually capable of initiating demyelination is not conclusive. Nevertheless, plasma exchange and immunosuppression with cyclophosphamide or other agents have produced clear-cut remissions with reversal of conduction block and disappearance of the paraprotein from the serum in some, though not all, of these patients. The role of immune cells in this disorder has not been determined, although mononuclear cell infiltrates are often present in demyelinated areas of peripheral nerve, and secretion of the IgM paraprotein appears to be under T cell control. This disorder is not only immunologically fascinating in its own right but may serve as a model for studies of other more common immune-mediated diseases of the peripheral and central nervous systems.

SLOW, CHRONIC, & LATENT VIRAL INFECTIONS OF THE NERVOUS SYSTEM

Several subacute and chronic degenerative diseases of human and animal nervous systems may be related to persistent or chronic infection of the nervous system with viruses and other less well defined transmissible agents. Subacute sclerosing panencephalitis in humans and a similar disorder produced experimentally in hamsters are related to persistent infection of the central nervous system with measles virus. Progressive multifocal leukoencephalopathy in humans has been associated with the recovery of 2 different papovaviruses previously thought not to be pathogenic for humans. A third group of diseases, referred to as the subacute spongiform encephalopathies, including kuru and Jakob-Creutzfeldt disease in humans and scrapie and mink encephalopathy in animals, have been shown to be caused by small transmissible agents that are present in affected brain material. These agents will produce an identical disease when inoculated into susceptible primate or nonprimate hosts after a relatively long disease-free latent period. Although the role of the host immune response is unclear in most of these disorders, the infectious nature of these diseases has directed research toward understanding the host response in both the treatment and possible prevention of these diseases.

Subacute Sclerosing Panencephalitis (SSPE)

Subacute sclerosing panencephalitis is a subacute degenerative central nervous system disease that can occur several years after acute infection with measles virus and presents mainly in school-age children. In most countries where an active measles vaccination program has been implemented, the incidence of SSPE has declined. The manifestations of the disease include personality change, dementia, seizures, and myoclonus which progress rapidly to death in 12–18 months in most cases. Pathologically, intranuclear and intracytoplasmic inclusion bodies had been noted for many years before electron microscopic studies revealed paramyxoviruslike structures in infected brain material. Later, measles virus antigen was demonstrated by fluorescent antibody staining and measles virus was recovered from infected brain material by co-cultivation techniques.

The persistence of measles virus in the presence of high titers of locally produced antimeasles antibody suggested that an underlying defect in immunity was present in patients with SSPE. However, consistent abnormalities in in vitro assays of cellular and humoral immunity could not be demonstrated. The possibility that measles virus associated with SSPE was different from wild-type measles virus was also entertained, but to date no biochemical or biologic marker that will differentiate an SSPE strain from a wild-type measles virus strain has been identified. Current evidence suggests that an abortive infection occurs and that mature virions are not produced. Incomplete expression or a defect in function of the matrix protein may be responsible. The accumulation of measles virus components within brain cells may alter cellular function, resulting in the appearance of the signs and symptoms associated with SSPE. Treatment with the antiviral agent isoprinosine or with interferon by intraventricular injection has resulted in stabilization and even improvement in some cases.

Progressive Multifocal Leukoencephalopathy (PML)

Progressive multifocal leukoencephalopathy is a disease of adults that presents as a widespread disease of the nervous system with ataxia, spasticity, visual disturbances, difficulty with speech and swallowing, and rapid progression to coma and death over a period of 1 year. The disease occurs most frequently in patients with debilitating illnesses, most of which produce some form of immunosuppression. These include lymphomas, leukemia, sarcoidosis, SLE, exogenous immunosuppression for renal transplantation, and acquired immunodeficiency syndrome (AIDS). Papovaviruses of 2 types, JC virus and SV40 virus, have been isolated and identified immunologically by the reaction of these viruses with specific antisera produced in rabbits. How these viruses are introduced into humans is unknown, but there was inadvertent introduction of SV40 virus by contaminated killed poliovirus vaccines in a large number of

people inoculated subcutaneously between 1955 and 1961, and there is some evidence of antibodies to SV40 virus in USA residents with no history of receiving contaminated poliovirus vaccine. Recently, a variable decrease in mitogen responsiveness and a specific absence of production of leukocyte inhibitory factor (LIF) in response to JC virus has been described in patients with progressive multifocal leukoencephalopathy. The added insult of generalized immunosuppression coupled with an absent or diminished response to latent papovaviruses may be involved in the pathogenesis of this disease.

Subacute Spongiform Encephalopathies

The neuropathologic similarities of kuru and Jakob-Creutzfeldt disease in humans and scrapie and mink encephalopathy in animals, as well as the fact that all are transmissible to other animals, have led to the suggestion by Gajdusek and Gibbs that these diseases should be considered similar types of subacute viral encephalopathies. The unconventional agents that cause these diseases have some viruslike properties; however, the illnesses are chronic and progressive after a symptom-free latent period, and no signs of an acute viral encephalitis occur. Clinically, there is no elevation of cerebrospinal fluid protein or γ-globulin and no detectable cellular response. Pathologically, there is no inflammatory reaction in the central nervous system. The pathologic changes consist of severe gliosis, loss of neurons in affected areas, and vacuolization within neuroglia. These changes are similar in naturally occurring illness and in experimentally transmitted disease.

Kuru was first described in 1957 by Gajdusek and Zigas in the Fore linguistic group of the New Guinea highlands. The disease presented as a subacute degenerative disorder characterized by progressive ataxia, tremor, dysphagia, and death within 3–18 months following the onset of symptoms. It had been noted that cannibalism was extensively practiced in this area, but its significance in terms of this disease was not appreciated until 1966, when it was demonstrated that kuru could be transmitted to chimpanzees by intracerebral inoculation of brain tissue from affected patients. It was later demonstrated that transmission could also occur by inoculation via the intradermal, intravenous, and oral routes not only with brain material but occasionally by inoculation of kidney, spleen, and lymph node material from affected patients. Since cannibalism was suppressed in this area in the 1950s the incidence of kuru has decreased.

Jakob-Creutzfeldt disease has been known clinically and pathologically for many years. The striking neuropathologic similarity to kuru and the rapid clinical course led to the attempted transmission of the disease to monkeys, which was successful after an incubation period of 13 months. Since the original transmission experiment Gibbs and Gajdusek have successfully transmitted the disease to several subhuman primates.

Scrapie, a naturally occurring disease of sheep and occasionally of goats, has been known since 1732. It was first described as transmissible in 1896, and this was confirmed in 1936. Later, the disease was transmitted to mice as well as monkeys after prolonged latent periods. The fact that the disease can be transmitted to mice makes it readily accessible to experimental study.

The natural course of scrapie in the mouse has been well characterized. The agent multiplies and reaches a relatively high titer in the spleen and lymphoid tissue 3–4 weeks after intracerebral and intravenous inoculation. The titer begins to fall thereafter in these organs but increases in the brain, where it reaches its peak at 6 months. The animals succumb to the disease 6–10 months following inoculation. There are no pathologic changes in the spleen or lymph nodes, but the nervous system does show spongiform changes prior to the onset of clinical symptoms. A protein called prion protein (Pr-P 27–30), encoded by a gene found in normal and scrapie-infected brain, has been associated with infectivity. Heterologous antiserum to Pr-P 27–30 raised in rabbits will react with rod-shaped and filamentous structures found in concentrated preparations of scrapie- and Jakob-Creutzfeldt disease-infected brains. Differences in the susceptibility of different strains of mice to scrapie and Jakob-Creutzfeldt disease which are linked to the H-2 complex and differences in susceptibility to kuru, scrapie, and Jakob-Creutzfeldt disease among monkeys suggest that certain genes may enhance the expression of the disease. However, the role of the immune response and its genetic regulation in these diseases is unclear. No antibodies to the infecting agent, evidence of immune paralysis, or disease-altering effects of immune system manipulation have been observed. These findings suggest that tolerance to an exogenous or aberrantly activated endogenous agent may be pathogenic features of these diseases. Autoantibodies directed against components of neurofilament proteins have been detected in the serum of patients with Jakob-Creutzfeldt disease and kuru and of animals with scrapie. Since they can also be identified in a smaller percentage of patients with nonviral neurodegenerative diseases, their presence probably represents a response to the neurodegeneration process initiated by the unconventional agents.

NEUROLOGIC DISEASES ASSOCIATED WITH THE ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Opportunistic infections of the central nervous system are a common complication of AIDS. A clinical syndrome of subacute encephalopathy and dementia associated with microglial clusters and monocyte-

derived giant cells in brain has also been described. Direct viral isolation, transmission of virus from infected brain, and in situ hybridization have demonstrated that HTLV-III/LAV/ARV, the agent responsible for AIDS, has neurotropic properties and accounts for many of the neurologic manifestations. In addition to dementia, myelopathy, peripheral neuropathy, and motor neuron disease have all been associated with AIDS.

The mechanisms by which HTLV-III/LAV/ARV produces nervous system damage are unclear. The paucity of pathologic findings and limited expression of viral antigens suggests an indirect effect. This appears to be the case in murine retrovirus-induced neurodegeneration, where virus replication occurs primarily in endothelial cells while neurons in adjacent areas demonstrate degenerative disease in the absence of virions and viral antigen. The presence of HTLV-III/LAV/ARV in brain may serve as a significant viral reservoir, and antiviral chemotherapy should be directed at elimination of this focus. Current research efforts utilizing molecular and nervous system culture techniques may shed new light on this perplexing problem.

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33 Eye Diseases

Mitchell H. Friedlaender, MD, & G. Richard O'Connor, MD

The eye is frequently considered to be a special target of immunologic disease processes, but proof of the causative role of these processes is lacking in all but a few disorders. In this sense, the immunopathology of the eye is much less clearly delineated than that of the kidney, the testis, or the thyroid gland. Because the eye is a highly vascularized organ and because the rather labile vessels of the conjunctiva are embedded in a nearly transparent medium, inflammatory eye disorders are more obvious (and often more painful) than those of other organs such as the thyroid or the kidney. The iris, ciliary body, and choroid are the most highly vascularized tissues of the eye. The similarity of the vascular supply of the uvea to that of the kidney and the choroid plexus of the brain has given rise to justified speculation concerning the selection of these 3 tissues, among others, as targets of immune complex diseases (eg, serum sickness).

Immunologic diseases of the eye can be grossly divided into 2 major categories: antibody-mediated and cell-mediated diseases. As is the case in other organs, there is ample opportunity for the interaction of these 2 systems in the eye.

ANTIBODY-MEDIATED DISEASES

Before it can be concluded that a disease of the eye is antibody-dependent, the following criteria must be satisfied: (1) There must be evidence of specific antibody in the patient's serum or plasma cells. (2) The antigen must be identified and, if feasible, characterized. (3) The same antigen must be shown to produce an immunologic response in the eye of an experimental animal, and the pathologic changes produced in the experimental animal must be similar to those observed in the human disease. (4) It must be possible to produce similar lesions in animals passively sensitized with serum from an affected animal upon challenge with the specific antigen.

Unless all of the above criteria are satisfied, the disease may be thought of as *possibly* antibody-dependent. In such circumstances, the disease can be regarded as antibody-mediated if only one of the following criteria is met: (1) if antibody to an antigen is present in higher quantities in the ocular fluids than in the serum (after adjustments have been made for the total amounts of immunoglobulins in each fluid); (2) if abnormal accumulations of plasma cells are present in the ocular lesion; (3) if abnormal accumulations of immunoglobulins are present at the site of the disease; (4) if complement is fixed by immunoglobulins at the site of the disease; (5) if an accumulation of eosinophils is present at the site of the disease; or (6) if the ocular disease is associated with an inflammatory disease elsewhere in the body for which antibody dependency has been proved or strongly suggested.

HAY FEVER CONJUNCTIVITIS

This disease is characterized by edema and hyperemia of the conjunctiva and lids (Fig 33–1) and by itching and watering of the eyes. There is often an associated itching sensation in the nose and rhinorrhea. The conjunctiva appears pale and boggy because of the intense edema, which is often rapid in onset. There is a distinct seasonal incidence, some patients being able to establish the onset of their symptoms at precisely the same time each year. These times usually correspond to the release of pollens by specific grasses, trees, or weeds.

Immunologic Pathogenesis

Hay fever conjunctivitis is one of the few inflammatory eye disorders for which antibody dependence has been definitely established. It is recognized as a form of atopic disease with an implied hereditary susceptibility. IgE (reaginic antibody) in its dimeric form is believed to be attached to mast cells lying beneath the conjunctival epithelium. Contact of the offending antigen with IgE triggers the release of vasoactive amines, principally histamine, in this area, and this in turn results in vasodilation and chemosis.

The role of circulating antibody to ragweed pollen in the pathogenesis of hay fever conjunctivitis has been demonstrated by passively transferring serum from a hypersensitive person to a nonsensitive one. When exposed to the offending pollen, the previously nonsensitive individual reacted with the typical signs of hay fever conjunctivitis.

Immunologic Diagnosis

Victims of hay fever conjunctivitis may show

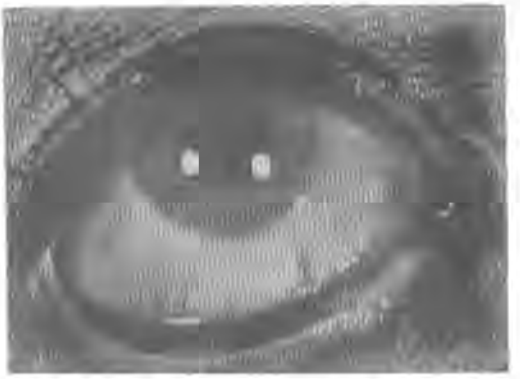

Figure 33–1. Hay fever conjunctivitis. Note edema and hyperemia of the conjunctiva. (Courtesy of M Allansmith and B McClellan.)

eosinophils in Giemsa-stained scrapings of conjunctival epithelium. They show the **immediate** type of response, with wheal and flare, when tested by scratch tests of the skin with extracts of pollens or other offending antigens. Biopsies of the skin test sites have occasionally shown the full-blown picture of an **Arthus reaction,** with deposition of immune complexes in the walls of the dermal vessels. Passive cutaneous anaphylaxis can also be used to demonstrate the presence of circulating antibody.

Treatment

Systemically administered antihistaminics such as diphenhydramine or tripelennamine are effective, particularly when given prophylactically during the season of greatest exposure. Sustained release capsules of antihistaminics such as Ornade (chlorpheniramine maleate) are preferred by some. Locally applied antihistaminics such as Prefrin-A drops contain both an antihistaminic agent (pyrilamine) and a vasoconstrictor (phenylephrine). Where conjunctival edema is severe and of sudden onset, epinephrine drops (1:100,000) instilled into the conjunctival sac may help to reduce the edema quickly. Corticosteroids applied locally offer some relief. Topical use of cromolyn (cromolyn sodium; Opticrom), a stabilizer of the mast cell, appears to be a promising method of treatment for many ocular allergic conditions.

Immunotherapy with gradually increasing doses of subcutaneously injected pollen extracts or other suspected allergens appears to reduce the severity of the disease in some individuals if started well in advance of the season. The mechanism is presumed to be production of blocking antibodies in response to the injection of small, graded doses of the antigen. This procedure cannot be recommended routinely, however, in view of the generally good results and relatively few complications of antihistamine therapy. Acute anaphylactoid reactions have occasionally resulted from overzealous immunotherapy.

VERNAL CONJUNCTIVITIS & ATOPIC KERATOCONJUNCTIVITIS

These 2 diseases also belong to the group of atopic disorders. Both are characterized by itching and lacrimation of the eyes but are more chronic than hay fever conjunctivitis. Furthermore, both ultimately result in structural modifications of the lids and conjunctiva.

Vernal conjunctivitis characteristically affects children and adolescents; the incidence decreases sharply after the second decade of life. Like hay fever conjunctivitis, vernal conjunctivitis occurs only in the warm months of the year. Most of its victims live in hot, dry climates. The disease characteristically produces giant ("cobblestone") papillae of the tarsal conjunctiva (Fig 33–2). The keratinized epithelium from these papillae may abrade the underlying cornea, giving rise to complaints of foreign body sensations.

Atopic keratoconjunctivitis affects individuals of all ages and has no specific seasonal incidence. The skin of the lids has a characteristic dry, scaly appearance. The conjunctiva is pale and boggy. Both the conjunctiva and the cornea may develop scarring in the later stages of the disease. Atopic cataract has also been described. Staphylococcal blepharitis, manifested by scales and crusts on the lids, commonly complicates this disease.

Immunologic Pathogenesis

Reaginic antibody (IgE) is fixed to subepithelial mast cells in both of these conditions. Contact between the offending antigen and IgE is thought to trigger degranulation of the mast cell, which in turn allows for the release of vasoactive amines in the tissues. It is unlikely, however, that antibody action alone is responsible, since—at least in the case of the papillae of vernal conjunctivitis—there is heavy papil-

Figure 33–2. Giant papillae ("cobblestones") in the tarsal conjunctiva of a patient with vernal conjunctivitis.

lary infiltration by mononuclear cells. Hay fever and asthma occur much more frequently in patients with vernal conjunctivitis and atopic keratoconjunctivitis than in the general population. Of the criteria outlined above (see p 610) for demonstration of *possibly* antibody-mediated diseases, (2), (5), and (6) have been met by atopic keratoconjunctivitis.

Immunologic Diagnosis

As in hay fever conjunctivitis, patients with atopic keratoconjunctivitis and vernal conjunctivitis regularly show large numbers of eosinophils in conjunctival scrapings. Skin testing with food extracts, pollens, and various other antigens reveals a wheal-and-flare reaction within 1 hour of testing, but the significance of these reactions is not reliably established.

Treatment

Local instillations of corticosteroid drops or ointment relieve the symptoms. However, caution must be observed in the long-term use of these agents because of the possibility of steroid-induced glaucoma and cataract. Corticosteroids produce less dramatic relief in vernal conjunctivitis than in atopic keratoconjunctivitis, and the same can be said of the antihistamines. Cromolyn seems to be useful in treating both atopic and vernal conjunctivitis and may allow the dosage of corticosteroids to be reduced.

Avoidance of known allergens is helpful; such objects as duck feathers, animal danders, and certain food proteins (egg albumin and others) are common offenders. Specific allergens have been much more difficult to demonstrate in the case of vernal disease, although some workers feel that such substances as rye grass pollens may play a causative role. Installation of air conditioning in the home or relocation to a cool, moist climate is useful in vernal conjunctivitis if economically feasible.

RHEUMATOID DISEASES AFFECTING THE EYE

The diseases in this category vary greatly in their clinical manifestations depending upon the specific disease entity and the age of the patient. Uveitis and scleritis are the principal ocular manifestations of the rheumatoid diseases. **Juvenile rheumatoid arthritis** affects females more frequently than males and is commonly accompanied by iridocyclitis of one or both eyes. The onset is often insidious, the patient having few or no complaints and the eye remaining white. Extensive synechia formation, cataract, and secondary glaucoma may be far-advanced before the parents notice that anything is wrong. The arthritis generally affects only one joint (eg, a knee) in cases with ocular involvement.

Ankylosing spondylitis affects males more frequently than females, and the onset is in the second to sixth decades. It may be accompanied by iridocyclitis of acute onset, often with fibrin in the anterior chamber (Fig 33–3). Pain, redness, and photophobia are the initial complaints, and synechia formation is common.

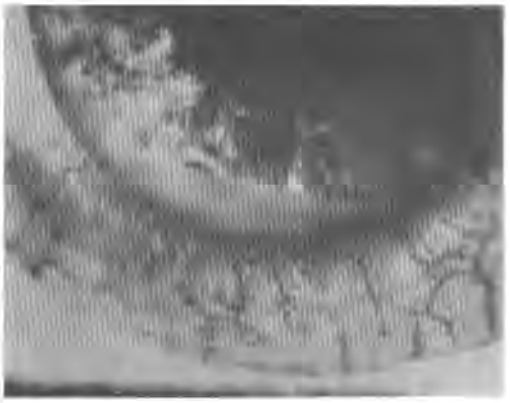

Figure 33–3. Acute iridocyclitis in a patient with ankylosing spondylitis. Note fibrin clot in anterior chamber.

Rheumatoid arthritis of adult onset may be accompanied by acute scleritis or episcleritis (Fig 33–4). The ciliary body and choroid, lying adjacent to the sclera, are often involved secondarily with the inflammation. Rarely, serous detachment of the retina results. The onset is usually in the third to fifth decade, and women are affected more frequently than men.

Reiter's disease affects men more frequently than women. The first attack of ocular inflammation usually consists of a self-limited papillary conjunctivitis. It follows, at a highly variable interval, the onset of nonspecific urethritis and the appearance of inflammation in one or more of the weight-bearing joints. Subsequent attacks of ocular inflammation may consist of acute iridocyclitis of one or both eyes, occasionally with hypopyon (Fig 33–5).

Immunologic Pathogenesis

Rheumatoid factor, an IgM autoantibody directed

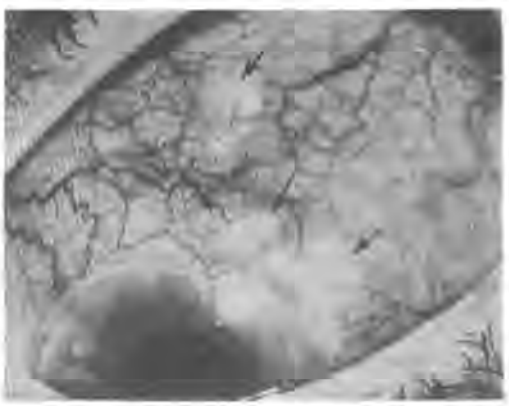

Figure 33–4. Scleral nodules in a patient with rheumatoid arthritis. (Courtesy of S Kimura.)

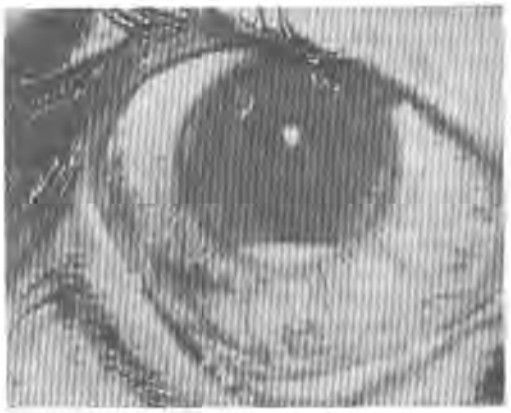

Figure 33–5. Acute iridocyclitis with hypopyon in a patient with Reiter's disease.

against the patient's own IgG, may play a major role in the pathogenesis of rheumatoid arthritis. The union of IgM antibody with IgG is followed by fixation of complement at the tissue site and the attraction of leukocytes and platelets to this area. An occlusive vasculitis resulting from this train of events is thought to be the cause of rheumatoid nodule formation in the sclera as well as elsewhere in the body. The occlusion of vessels supplying nutriments to the sclera is thought to be responsible for the "melting away" of the scleral collagen that is so characteristic of rheumatoid arthritis (Fig 33–6).

While this explanation may suffice for rheumatoid arthritis, patients with the ocular complications of juvenile rheumatoid arthritis, ankylosing spondylitis, and Reiter's syndrome usually have negative tests for rheumatoid factor, so other explanations must be sought.

Outside the eyeball itself, the lacrimal gland has been shown to be under attack by circulating antibodies. Destruction of acinar cells within the gland and invasion of the lacrimal gland (as well as the salivary glands) by mononuclear cells result in decreased tear secretion. The combination of dry eyes (keratoconjunctivitis sicca), dry mouth (xerostomia), and rheumatoid arthritis is known as Sjögren's syndrome (see Chapters 21 and 36).

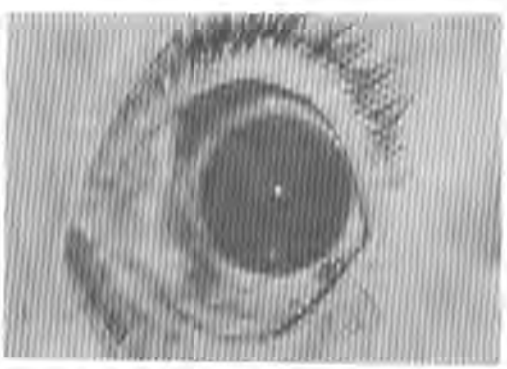

Figure 33–6. Scleral thinning in a patient with rheumatoid arthritis. Note dark color of the underlying uvea.

A growing body of evidence indicates that the immunogenetic background of certain patients accounts for the expression of their ocular inflammatory disease in specific ways. Analysis of the HLA antigen system shows that the incidence of HLA-B27 is significantly greater in patients with ankylosing spondylitis and Reiter's syndrome than could be expected by chance alone. It is not known how this antigen controls specific inflammatory responses.

Immunologic Diagnosis

Rheumatoid factor can be detected in the serum by a number of standard tests involving the agglutination of IgG-coated erythrocytes or latex particles. Unfortunately, the test for rheumatoid factor is not positive in the majority of isolated rheumatoid afflictions of the eye.

The HLA types of individuals suspected of having ankylosing spondylitis and related diseases can be determined by standard cytotoxicity tests using highly specific antisera. This is generally done in tissue typing centers where work on organ transplantation necessitates such studies. X-ray of the sacroiliac area is a valuable screening procedure that may show evidence of spondylitis prior to the onset of low back pain in patients with the characteristic form of iridocyclitis.

Treatment

Patients with uveitis associated with rheumatoid disease respond well to local instillations of corticosteroid drops (eg, dexamethasone 0.1%) or ointments. Orally administered corticosteroids must occasionally be resorted to for brief periods. Salicylates given orally in divided doses with meals are thought to reduce the frequency and blunt the severity of recurrent attacks. Atropine drops (1%) are useful for the relief of photophobia during the acute attacks. Shorter-acting mydriatics such as phenylephrine 10% should be used in the subacute stages to prevent synechia formation. Corticosteroid-resistant cases, especially those causing progressive erosion of the sclera, have been treated successfully with immunosuppressive agents such as chlorambucil.

OTHER ANTIBODY-MEDIATED EYE DISEASES

The following antibody-mediated diseases are infrequently seen by the practicing ophthalmologist.

Systemic lupus erythematosus, associated with the presence of circulating antibodies to DNA, produces an occlusive vasculitis of the nerve fiber layer of the retina. Such infarcts result in cytoid bodies or "cotton wool" spots in the retina (Fig 33–7).

Pemphigus vulgaris produces painful intraepithelial bullae of the conjunctiva It is associated

Figure 33-7. "Cotton wool" spots in the retina of a patient with lupus erythematosus.

with the presence of circulating antibodies to an intercellular antigen located between the deeper cells of the conjunctival epithelium.

Cicatricial pemphigoid is characterized by subepithelial bullae of the conjunctiva. In the chronic stages of this disease, cicatricial contraction of the conjunctiva may result in severe scarring of the cornea, dryness of the eyes, and ultimate blindness. Pemphigoid is associated with local deposits of tissue antibodies directed against one or more antigens located in the basement membrane of the epithelium.

Lens-induced uveitis is a rare condition that may be associated with circulating antibodies to lens proteins. It is seen in individuals whose lens capsules have become permeable to these proteins as a result of trauma or other disease. Interest in this field dates back to 1903, when Uhlenhuth first demonstrated the organ-specific nature of antibodies to the lens. Witmer showed in 1962 that antibody to lens tissue may be produced by lymphoid cells of the ciliary body.

CELL-MEDIATED DISEASES

This group of diseases appears to be associated with cell-mediated immunity or delayed hypersensitivity. Various structures of the eye are invaded by mononuclear cells, principally lymphocytes and macrophages, in response to one or more chronic antigenic stimuli. In the case of chronic infections such as tuberculosis, leprosy, toxoplasmosis, and herpes simplex, the antigenic stimulus has clearly been identified as an infectious agent in the ocular tissue. Such infections are often associated with delayed skin test reactivity following the intradermal injection of an extract of the organism.

More intriguing but less well understood are the granulomatous diseases of the eye for which no infectious cause has been found. Such diseases are thought to represent cell-mediated, possibly autoimmune processes, but their origin remains obscure.

OCULAR SARCOIDOSIS

Ocular sarcoidosis is characterized by a panuveitis with occasional inflammatory involvement of the optic nerve and retinal blood vessels. It often presents as iridocyclitis of insidious onset. Less frequently, it occurs as acute iridocyclitis, with pain, photophobia, and redness of the eye. Large precipitates resembling drops of solidified "mutton fat" are seen on the corneal endothelium. The anterior chamber contains a good deal of protein and numerous cells, mostly lymphocytes. Nodules are often seen on the iris, both at the pupillary margin and in the substance of the iris stroma. The latter are often vascularized. Synechiae are commonly encountered, particularly in patients with dark skin. Severe cases ultimately involve the posterior segment of the eye. Coarse clumps of cells ("snowballs") are seen in the vitreous, and exudates resembling candle drippings may be seen along the course of the retinal vessels. Patchy infiltrations of the choroid or optic nerve may also be seen.

Infiltrations of the lacrimal gland and of the conjunctiva have been noted on occasion. When the latter are present, the diagnosis can easily be confirmed by biopsy of the small opaque nodules.

Immunologic Pathogenesis

Although many infectious or allergic causes of sarcoidosis have been suggested, none has been confirmed. Noncaseating granulomas are seen in the uvea, optic nerve, and adnexal structures of the eye as well as elsewhere in the body. The presence of macrophages and giant cells suggests that particulate matter is being phagocytized, but this material has not been identified.

Patients with sarcoidosis are usually anergic to extracts of the common microbial antigens such as those of mumps, *Trichophyton*, *Candida*, and *Mycobacterium tuberculosis*. As in other lymphoproliferative disorders such as Hodgkin's disease and chronic lymphocytic leukemia, this may represent suppression of T cell activity such that the normal delayed hypersensitivity responses to common antigens cannot take place. Meanwhile, circulating immunoglobulins are usually detectable in the serum at higher than normal levels.

Immunologic Diagnosis

The diagnosis is largely inferential. Negative skin tests to a battery of antigens to which the patient is known to have been exposed are highly suggestive, and the same is true of the elevation of serum immunoglobulins. Biopsy of a conjunctival nodule or scalene lymph node may provide positive histologic evidence of the disease. X-rays of the chest reveal hilar adenopathy in many cases. Elevated levels of

serum lysozyme or serum angiotensin-converting enzyme may be detected. A gallium scan, utilizing ^{67}gallium, may be useful in detecting clinically inapparent lesions.

Treatment

Sarcoid lesions of the eye respond well to corticosteroid therapy. Frequent instillations of prednisolone acetate 1% eye drops generally bring the anterior uveitis under control. Atropine drops should be prescribed in the acute phase of the disease for the relief of pain and photophobia; short-acting pupillary dilators such as phenylephrine should be given later to prevent synechia formation. Systemic corticosteroids are sometimes necessary to control severe attacks of anterior uveitis and are always necessary for the control of retinal vasculitis and optic neuritis. The latter condition often accompanies cerebral involvement and carries a grave prognosis.

SYMPATHETIC OPHTHALMIA & VOGT-KOYANAGI-HARADA SYNDROME

These 2 disorders are discussed together because they have certain common clinical features. Both are thought to represent autoimmune phenomena affecting pigmented structures of the eye and skin, and both may give rise to meningeal symptoms.

Clinical Features

Sympathetic ophthalmia is an inflammation in the second eye after the other has been damaged by penetrating injury. In most cases, some portion of the uvea of the injured eye has been exposed to the atmosphere for at least 1 hour. The uninjured or "sympathizing" eye develops minor signs of anterior uveitis after a period ranging from 2 weeks to several years. Floating spots and loss of the power of accommodation are among the earliest symptoms. The disease may progress to severe iridocyclitis with pain and photophobia. Usually, however, the eye remains relatively quiet and painless while the inflammatory disease spreads around the entire uvea. Despite the presence of panuveitis, the retina usually remains uninvolved except for perivascular cuffing of the retinal vessels with inflammatory cells. Papilledema and secondary glaucoma may occur. The disease may be accompanied by vitiligo (patchy depigmentation of the skin) and poliosis (whitening) of the eyelashes.

Vogt-Koyanagi-Harada syndrome consists of inflammation of the uvea of one or both eyes characterized by acute iridocyclitis, patchy choroiditis, and serous detachment of the retina. It usually begins with an acute febrile episode with headache, dysacusis, and occasionally vertigo. Patchy loss or whitening of the scalp hair is described in the first few months of the disease. Vitiligo and poliosis are commonly present but are not essential for the diagnosis. Although the initial iridocyclitis may subside quickly, the course of the posterior disease is often indolent, with long-standing serous detachment of the retina and significant visual impairment.

Immunologic Pathogenesis

In both sympathetic ophthalmia and Vogt-Koyanagi-Harada syndrome, delayed hypersensitivity to melanin-containing structures is thought to occur. Although a viral cause has been suggested for both disorders, there is no convincing evidence of an infectious origin. It is postulated that some insult, infectious or otherwise, alters the pigmented structures of the eye, skin, and hair in such a way as to provoke delayed hypersensitivity responses to them. Soluble materials from the outer segments of the photoreceptor layer of the retina have recently been incriminated as possible autoantigens. Patients with Vogt-Koyanagi-Harada syndrome are usually Orientals, which suggests an immunogenetic predisposition to the disease.

Histologic sections of the traumatized eye from a patient with sympathetic ophthalmia may show uniform infiltration of most of the uvea by lymphocytes, epithelioid cells, and giant cells. The overlying retina is characteristically intact, but nests of epithelioid cells may protrude through the pigment epithelium of the retina, giving rise to **Dalen-Fuchs nodules.** The inflammation may destroy the architecture of the entire uvea, leaving an atrophic, shrunken globe.

Immunologic Diagnosis

Skin tests with soluble extracts of human or bovine uveal tissue are said to elicit delayed hypersensitivity responses in these patients. Several investigators have recently shown that cultured lymphocytes from patients with these 2 diseases undergo transformation to lymphoblasts in vitro when extracts of uvea or rod outer segments are added to the culture medium. Circulating antibodies to uveal antigens have been found in patients with these diseases, but such antibodies are to be found in any patient with long-standing uveitis, including those suffering from several infectious entities. The spinal fluid of patients with Vogt-Koyanagi-Harada syndrome may show increased numbers of mononuclear cells and elevated protein in the early stages.

Treatment

Mild cases of sympathetic ophthalmia may be treated satisfactorily with locally applied corticosteroid drops and pupillary dilators. The more severe or progressive cases require systemic corticosteroids, often in high doses, for months or years. An alternate-day regimen of oral corticosteroids is recommended for such patients in order to avoid adrenal suppression. The same applies to the treatment of patients with Vogt-Koyanagi-Harada disease. Occasionally, patients with long-standing progressive disease become resistant to corticosteroids or cannot take additional corticosteroid medication because of pathologic fractures, mental changes, or other reasons. Such patients may become candidates for immunosuppressive ther-

apy. Chlorambucil and cyclophosphamide have been used successfully for both conditions. More recently, cyclosporine has shown promise in the treatment of corticosteroid-resistant uveitis.

OTHER DISEASES OF CELL-MEDIATED IMMUNITY

Giant cell arteritis (temporal arteritis) (see Chapter 21) may have disastrous effects on the eye, particularly in elderly individuals. The condition is manifested by pain in the temples and orbit, blurred vision, and scotomas. Examination of the fundus may reveal extensive occlusive retinal vasculitis and choroidal infarcts. Atrophy of the optic nerve head is a frequent complication. Such patients have an elevated sedimentation rate. Biopsy of the temporal artery reveals extensive infiltration of the vessel wall with giant cells and mononuclear cells.

Polyarteritis nodosa (see Chapter 21) can affect both the anterior and posterior segments of the eye. The corneas of such patients may show peripheral thinning and cellular infiltration. The retinal vessels reveal extensive necrotizing inflammation characterized by eosinophil, plasma cell, and lymphocyte infiltration.

Behçet's disease (see Chapter 21) has an uncertain place in the classification of immunologic disorders. It is characterized by recurrent iridocyclitis with hypopyon and occlusive vasculitis of the retinal vessels. Although it has many of the features of a delayed hypersensitivity disease, dramatic alterations of serum complement levels at the very beginning of an attack suggest an immune complex disorder. Furthermore, high levels of circulating immune complexes have recently been detected in patients with this disease. Most patients with eye symptoms are positive for HLA-B5 (subtype B51).

Contact dermatitis of the eyelids represents a significant though minor disease caused by delayed hypersensitivity. Atropine, perfumed cosmetics, materials contained in plastic spectacle frames, and other locally applied agents may act as the sensitizing hapten. The lower lid is more extensively involved than the upper lid when the sensitizing agent is applied in drop form. Periorbital involvement with erythematous, vesicular, pruritic lesions of the skin is characteristic.

Phlyctenular keratoconjunctivitis (Fig 33-8) represents a delayed hypersensitivity response to certain microbial antigens, principally those of *Mycobacterium tuberculosis*. It is characterized by acute pain and photophobia in the affected eye, and perforation of the peripheral cornea has been known to result from it. The disease responds rapidly to locally applied corticosteroids. Since the advent of chemotherapy for pulmonary tuberculosis, phlyctenulosis is much less of a problem than it was 30 years ago. It is still encountered occasionally, however, particularly among Native Americans and Alaskan Eskimos. Rarely, other pathogens such as *Staphylococcus aureus* and *Coccidioides immitis* have been implicated in phlyctenular disease.

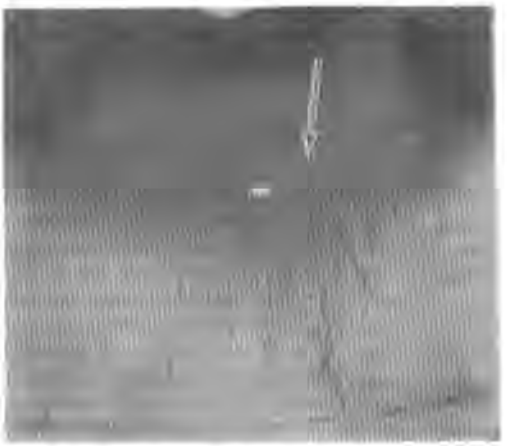

Figure 33–8. Phlyctenule (arrow) at the margin of the cornea. (Courtesy of P Thygeson.)

Acquired Immunodeficiency Syndrome (AIDS). Ocular disorders are a common feature of acquired immunodeficiency syndrome (AIDS), seen mainly in homosexual men, intravenous drug abusers, and hemophiliacs. Cotton wool exudates are the most common ocular sign. They have the same appearance as those seen in systemic lupus erythematosus (Fig 33–7), but it is not known whether the cotton wool spots of AIDS have the same pathogenesis. As is the case with SLE, patients suffering from AIDS may have elevated serum immune complexes.

In addition to cotton wool spots, AIDS patients may develop Kaposi's sarcoma of the conjunctiva or lids as well as chorioretinitis associated with any one of a number of different opportunistic pathogens such as cytomegalovirus, *Cryptococcus*, *Toxoplasma*, or *Candida*. These patients have a fundamental disorder of cell-mediated immunity reflected in abnormal ratios of helper T to suppressor T lymphocytes. The condition is due to a blood-borne virus (HTLV-III/LAV/ARV) that can be sexually transmitted. These patients often die of systemic opportunistic infections such as *Pneumocystis carinii* pneumonia or toxoplasmal encephalitis. Since cotton wool spots are an early sign of AIDS, the ophthalmologist may be the first physician to alert the patient to the existence of this serious disorder.

CORNEAL GRAFT REACTIONS

Blindness due to opacity or distortion of the central portion of the cornea is a remediable disease. If all other structures of the eye are intact, a patient whose vision is impaired solely by corneal opacity can expect great improvement from a graft of clear cornea into the

diseased area. Trauma, including chemical burns, is one of the most common causes of central corneal opacity. Others include scars from herpetic keratitis, endothelial cell dysfunction with chronic corneal edema (Fuchs's dystrophy), keratoconus, and opacities from previous graft failures. All of these conditions represent indications for penetrating corneal grafts, provided the patient's eye is no longer inflamed and the opacity has been allowed maximal time to undergo spontaneous resolution (usually 6–12 months). It is estimated that approximately 10,000 corneal grafts are performed in the USA annually. Of these, about 90% can be expected to produce a beneficial result.

The cornea was one of the first human tissues to be successfully grafted. The fact that recipients of corneal grafts generally tolerate them well can be attributed to (1) the absence of blood vessels or lymphatics in the normal cornea and (2) the lack of presensitization to tissue-specific antigens in most recipients. Reactions to corneal grafts do occur, however, particularly in individuals whose own corneas have been damaged by previous inflammatory disease. Such corneas may have developed both lymphatics and blood vessels, providing afferent and efferent channels for immunologic reactions in the engrafted cornea.

Although attempts have been made to transplant corneas from other species into human eyes (xenografts), particularly in countries where human material is not available for religious reasons, most corneal grafts have been taken from human eyes (allografts). Except in the case of identical twins, such grafts always represent the implantation of foreign tissue into a donor site; thus, the chance for a graft rejection due to an immune response to foreign antigens is virtually always present.

The cornea is a 3-layered structure composed of a surface epithelium, an oligocellular collagenous stroma, and a single-layered endothelium. Although the surface epithelium may be sloughed and later replaced by the recipient's epithelium, certain elements of the stroma and all of the donor's endothelium remain in place for the rest of the patient's life. This has been firmly established by sex chromosome markers in corneal cells when donor and recipient were of opposite sexes. The endothelium must remain healthy in order for the cornea to remain transparent, and an energy-dependent pump mechanism is required to keep the cornea from swelling with water. Since the recipient's endothelium is in most cases diseased, the central corneal endothelium must be replaced by healthy donor tissue.

A number of foreign elements exist in corneal grafts that might stimulate the immune system of the host to reject this tissue. In addition to those mentioned above, the corneal stroma is regularly perfused with IgG and serum albumin from the donor, although none—or only small amounts—of the other blood proteins are present. While these serum proteins of donor origin rapidly diffuse into the recipient stroma, these substances are theoretically immunogenic.

Although the ABO blood antigens have been shown to have no relationship to corneal graft rejection, the HLA antigen system probably plays a significant role in graft reactions. HLA incompatibility between donor and recipient has been shown by several authors to be significant in determining graft survival, particularly when the corneal bed is vascularized. It is known that most cells of the body possess these HLA antigens, including the endothelial cells of the corneal graft as well as certain stromal cells (keratocytes). The epithelium has been shown by Hall and others to possess a non-HLA antigen that diffuses into the anterior third of the stroma. Thus, while much foreign antigen may be eliminated by purposeful removal of the epithelium at the time of grafting, that amount of antigen which has already diffused into the stroma is automatically carried over into the recipient. Such antigens may be leached out by soaking the donor cornea in tissue culture for several weeks prior to engraftment.

Both humoral and cellular mechanisms have been implicated in corneal graft reactions. It is likely that early graft rejections (within 2 weeks) are cell-mediated reactions. Cytotoxic lymphocytes have been found in the limbal area and stroma of affected individuals, and phase microscopy in vivo has revealed an actual attack on the grafted endothelial cells by these lympohocytes. Such lymphocytes generally move inward from the periphery of the cornea, making what is known as a "rejection line" as they move centrally. The donor cornea becomes edematous as the endothelium becomes compromised by an accumulation of lymphoid cells.

Late rejection of a corneal graft may occur several weeks to many months after implantation of donor tissue into the recipient eye. Such reactions may be antibody-mediated, since cytotoxic antibodies have been isolated from the serum of patients with a history of

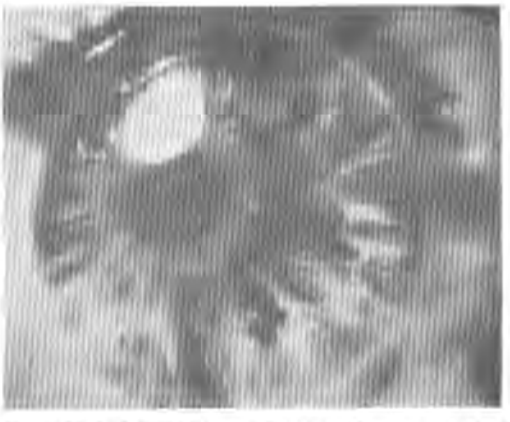

Figure 33–9. A cornea severely scarred by chronic atopic keratoconjunctivitis into which a central graft of clear cornea has been placed. Note how distinctly the iris landmarks are seen through the transparent graft.

multiple graft reactions in vascularized corneal beds. These antibody reactions are complement-dependent and attract polymorphonuclear leukocytes, which may form dense rings in the cornea at the sites of maximum deposition of immune complexes. In experimental animals, similar reactions have been produced by corneal xenografts, but the intensity of the reaction can be markedly reduced either by decomplementing the animal or by reducing its leukocyte population through mechlorethamine therapy.

Treatment

The mainstay of the treatment of corneal graft reactions is corticosteroid therapy. This medication is generally given in the form of frequently applied eye drops (eg, prednisolone acetate, 1%, hourly) until the clinical signs abate. These clinical signs consist of conjunctival hyperemia in the perilimbal region, a cloudy cornea, cells and protein in the anterior chamber, and keratic precipitates on the corneal endothelium. The earlier treatment is applied, the more effective it is likely to be. Neglected cases may require systemic or periocular corticosteroids in addition to local eye drop therapy. Occasionally, vascularization and opacification of the cornea occur so rapidly as to make corticosteroid therapy useless, but even the most hopeless-appearing graft reactions have occasionally been reversed by corticosteroid therapy.

Patients known to have rejected many previous corneal grafts are managed somewhat differently, particularly if disease affects their only remaining eye. An attempt is made to find a close HLA match between donor and recipient. Pretreatment of the recipient with immunosuppressive agents such as azathioprine has also been resorted to in some cases. Although HLA testing of the recipient and the potential donor is indicated in cases of repeated corneal graft failure or in cases of severe corneal vascularization, such testing is not necessary or practicable in most cases requiring keratoplasty.

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Reproductive Immunology

34

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The immune and reproductive systems interact at many levels. Impregnation of the female by sperm represents an intrusion that must be accepted in order to produce progeny. Fertilization itself must occur in a relatively hostile immunologic environment, since it involves intimate association of the histoincompatible sperm and ovum. Implantation of the zygote with its full complement of paternal and maternal genes and the subsequent development of the placenta are events that should theoretically evoke antagonistic maternal immune responses. However, gestation in all outbred species is a fact that appears to transgress the laws of transplantation. The parturition process has many similarities to immune rejection, but currently there is no evidence that birth is a consequence of the termination of immunologic tolerance between fetus and mother.

Infertility can be induced experimentally by immunization with sperm. Potentially adverse consequences of immunity to sperm in vasectomized males with autoimmunity to sperm is a subject of great interest. Seminal plasma constituents appear to be capable of regulating local immune responses that might otherwise be harmful. Derangements of these physiologic processes in which immune and reproductive systems interact can cause disease. New knowledge regarding pathologic events in this interaction will continue to provide insights into both reproduction and immunology.

REPRODUCTIVE IMMUNOLOGY IN THE FEMALE

Periodic preparations for fertilization and pregnancy occur at monthly intervals when the human female undergoes a physiologic cycle involving maturation and ovulation of an oocyte. The ovum exists temporarily in the reproductive tract unless fertilized by spermatozoa following copulation. After successful fertilization, various components of the uterine (fallopian) tubes begin to nourish and transport the zygote to the uterine cavity; and, upon maturation to the blastocyst stage, the embryo implants itself into the uterine endometrium. The outer layer of cells of the implanted blastocyst, the syncytiotrophoblast, which consists of a multinucleate mass without discernible cell boundaries, now begins to actively invade and deeply penetrate the neighboring uterine connective tissue and—together with embryonic cells derived from the inner cell mass—gives rise to the more complex fetoplacental unit. These events of viviparous reproduction represent a highly successful evolutionary process whereby the problems of transplant rejection response have been largely circumvented.

IMMUNOREGULATION OF MATERNAL RECOGNITION OF THE FETAL ALLOGRAFT

A central unresolved question is why the developing fetus, which possesses paternal transplantation antigens foreign to the mother and is therefore similar to an allograft, is able to implant and grow in the uterus. The evasion of immune destruction is even more striking, since both humoral and cellular components of the immune system of the pregnant female are activated presumably as a consequence of natural immunization induced by the embryo. Various mechanisms have been proposed to explain the protection of the fetus against expected immune destruction. These include (1) a physical or anatomic barrier (the trophoblast) that surrounds the fetus and prevents passage of maternal lymphoid cells; (2) lack of a full complement of immunogenic paternally derived histocompatibility antigens on the intervening layer of trophoblastic cells, which are thereby incapable of eliciting maternal immune effector mechanisms; and (3) suppressor activity by fetal lymphoid cells, placental cells, and hormones. Any or all of these could enable the fetus to inhibit or circumvent immunologic attack. Other possibilities such as the uterus as a privileged immunologic site and a nonspecific weakening of the maternal immune system during pregnancy have been largely discarded in recent years.

THE UTERUS AS A SITE FOR IMMUNE REACTIVITY

Although the uterus was once considered an immunologically privileged site, most current evidence indicates that both afferent and efferent limbs of the

immune response are operative in the area of this reproductive organ. Placement of experimental allografts of normal noninvasive tissue into the nonpregnant uterus usually results in their prompt rejection, similar to the fate of foreign skin grafts. Introduction of allogeneic epidermal cells, leukocytes, or spermatozoa into the uterus immunizes the recipient, resulting in hypertrophy of the draining para-aortic lymph nodes as well as a state of alloimmunity. It appears that antigenic material is capable of passing through the endometrium and is taken up by the neighboring lymphatic vessels. Washed rodent spermatozoa have a priming effect after instillation into the uterine cavity, whereas extrauterine placement is usually ineffective in evoking transplantation immunity. These examples of intrauterine sensitization against alloantigens using skin grafts or injections of cell suspensions can lead to a secondary response when there is a local challenge with tissue material of the same antigenic specificity. In addition, local alloimmunization in the uterine environment has a dramatic effect on subsequent reproductive capabilities. Increased numbers of embryos develop in pregnant animals whose uterine horns have been presensitized against paternal histocompatibility antigens and have already expressed the local "recall flare," a delayed hypersensitivity reaction. The immunologic basis for this unexpected result is unknown, although it suggests that maternal immune reactivity has a beneficial effect and may play a vital role in maternal-fetal coexistence.

Following sexual intercourse, allogeneic spermatozoa are not ordinarily recognized as foreign and are therefore not rejected in the immunocompetent maternal host. This phenomenon may be related to the presence of nonspecific immunosuppressive factors in semen. A high-molecular-weight component present in human seminal plasma has a strong suppressive effect on mitogen, antigen, and allogeneic cell activation of human lymphocytes, while other substances in semen have been shown to interfere with the microbicidal activity of antibody, complement, and granulocytes.

The events of implantation of the fertilized egg and ensuing invasion of uterine tissue by the trophoblast evoke an inflammatory reaction resulting in the formation of a highly specialized gestational tissue called the decidua. Besides possessing endocrinologic activity, decidual tissue may act as a selective barrier by preventing released fetal or trophoblastic antigens from reaching the neighboring afferent lymphatic vessels and by preventing access of sensitized maternal lymphocytes to the conceptus. The decidua may also release immunosuppressive factors as well as express NK-like activity very early during its development. Experimental induction of the decidual reaction affords increased survival of skin allografts that have been inserted in the uteri of pseudopregnant animals. The well-known fact that ectopic pregnancy can elicit decidual reactions in extrauterine sites suggests that this locally evoked reaction at the site of implantation and the later development of the placenta play key roles in maintaining the integrity of the fetus.

MATERNAL IMMUNE RESPONSE DURING PREGNANCY

During pregnancy, there is enlargement of the lymph nodes draining the uterus, presumably in response to foreign fetal antigens or to the protein or steroid hormones produced in relatively high concentrations by the fetoplacental unit. Maternal-fetal incompatibilities can stimulate immune responses in pregnant women, resulting in the production of anti-Rh antibodies in Rh-negative women and in the formation of antihistocompatibility antibodies. These maternal antibodies react relatively specifically with both fetal and paternal leukocyte antigens, thus enabling pregnancy sera to be used as a convenient source of HLA antibodies. This humoral response appears to be augmented with increasing parity. Antibodies also arise that agglutinate leukocytes, are cytotoxic to lymphocytes, and react with antigens contained in the cytoplasm of the syncytiotrophoblast. These pregnancy-induced antibodies have a wide range of activities in vitro. They are capable of inhibiting the mixed lymphocyte reaction and the production of MIF and can interfere with the killing of trophoblast cells by maternal lymphocytes. Antibodies in pregnancy sera that inhibit the mixed lymphocyte reaction are directed primarily against HLA antigens. Of clinical interest is the lack of correlation between the formation of anti-HLA antibodies by the mother and the incidence of congenital disorders.

Using monoclonal antibodies, some investigators have shown that during human pregnancy there is a slight reduction in the number of helper T cells and in the helper:suppressor cell ratio, while others have shown a lack of any numerical imbalance in these T cell subpopulations. It is likely that these alterations in T cell subsets, if they exist at all, are related to fluctuating hormone levels during gestation and do not necessarily correlate with any clinically significant changes in T cell function. Indeed, a significant number of women express cell-mediated immunity to fetoplacental antigens. When maternal and newborn lymphocytes are mixed together, the production of MIF for guinea pig macrophages was observed in 50% of the mothers studied immediately after delivery. MIF was not detected when maternal cells were cultured with lymphocytes from unrelated donors. In a larger study using the MIF assay, it was demonstrated that pregnant women develop cellular immunity against placental antigens during the fourth month of pregnancy, and this immunity can be maintained for several months. These experiments may measure the response to specific trophoblast antigens not present on cord blood lymphocytes. Human maternal lymphocytes can kill fetoplacental target cells in vitro. However, convincing evidence that this phenomenon occurs in other species is lacking or has been obscured because of inhibitors in pregnancy serum.

Another in vitro indicator of cellular immunity, the mixed lymphocyte reaction, measures allogeneic differences between 2 cell populations and correlates

well with the genetic disparity between fetus and mother. Lymphocyte responses of pregnant hosts to paternal or unrelated alloantigens in the mixed lymphocyte reaction do not seem to differ greatly when compared to the responses of nonpregnant controls. Similar results have been reported when responses to PHA were evaluated, indicating that maternal lymphocyte activity during pregnancy is functionally intact. However, substances present in pregnancy serum substantially alter these otherwise normal immune responses. Clinical studies have shown that these inhibitors were absent from the sera of women who were prone to idiopathic spontaneous abortions.

ALLOANTIGENICITY OF THE FETOPLACENTAL UNIT & AN IMMUNOLOGIC ROLE FOR THE PLACENTA

The placenta is a unique and complex organ by virtue of the brief duration of its biologic existence and the heterogeneous composition of structural elements and functionally active cell types belonging to the trophoblastic, lymphocytic, and erythroid series. Like the fetus, the placenta consists of tissue derived from 2 different parental genotypes. The production of protein and steroid hormones that regulate the physiologic activities of pregnancy has long been recognized as a crucial function of the placenta. Concurrently, it acts as the fetal lung, kidney, intestine, and liver. It is becoming increasingly evident that an immunologic role for this organ may be of paramount importance for the successful maintenance of mammalian pregnancy.

That both the maternal and paternal components of fetal transplantation antigens are expressed on cellular elements within the placenta seems unquestionable. However, from an immunologic standpoint, the key question is whether transplantation antigens can be demonstrated on trophoblast membranes. These membranes are the interface in direct apposition to the maternal circulation in hemochorial placentas (such as in humans). As such, they present a direct challenge for both the afferent and efferent limbs of the immune response and could serve as the site of immune attack by immunologically competent maternal lymphocytes.

Whether or not transplantation antigens are expressed on trophoblasts has become a matter of intense controversy. The answer seems to be dependent upon the ontogenetic and phylogenetic expression of these antigens at various stages of gestation. It has been reported that placental antigens may be masked by histocompatibility or specific trophoblast antibodies, fibrinoid, fibrinomucoid, or immune complexes. Class I (HLA-A, -B, -C) antigens can be detected on early human placental cytotrophoblast, although it has been difficult to demonstrate any HLA antigens on other human trophoblast tissue, including the syncytiotrophoblast of the mature chorionic villus. Serologic and transplantation studies show that H-2 antigens are expressed weakly on early-stage murine trophoblast, while significant levels of these antigens have been found on late gestational trophoblast material. In all cases, class I (H-2K/D) rather than class II (Ia) MHC antigens appear to be selectively expressed on the trophoblast. Since Ia antigens are known to play a major role in lymphocyte activation, the survival of the trophoblast could therefore be the direct result of the pregnant host's inability to mount an effective cell-mediated rejection type response against it. Whether transplantation antigens are present in an immunogenic form on mammalian trophoblast cells is an important key to our understanding of the many aspects of the immunologic interaction between mother and fetus. The demonstration that maternal lymphocytes are capable of killing cultured human trophoblast cells from their own placenta and that late gestational placental trophoblast cells from mice can induce specific cell-mediated immunity for the relevant paternal alloantigenic determinants or can serve as target cells in vitro for specific antipaternal cell-mediated cytotoxicity is further evidence that trophoblast cells do display transplantation antigens. Extrauterine placental allografts are usually rejected by allogeneic recipients and provoke a state of alloimmunity, while transplants of trophoblast from early gestational tissue proceed to grow and develop unimpeded without eliciting a detectable allograft rejection response.

A variety of hormonal and immunologic events occurring during pregnancy could modulate maternal transplantation immunity against paternal antigens expressed on the placenta and trophoblast. Circulating substances such as alpha-fetoprotein, placental-ovarian steroids, protein hormones, and antibody have widely different concentrations in pregnancy serum than at their sites of production. These factors have been proposed as naturally occurring immunosuppressive factors. However, there is currently no firm evidence that any of these circulating factors adequately explain the cell regulatory events that occur in the maternal immune system. Although the individual concentrations of a variety of gestational steroid and protein hormones in maternal serum never achieve a level that will suppress immunity in vivo, these substances taken together could exert a potent immunosuppressive effect at the fetal-maternal interface, where they are made and maintained at high levels throughout most stages of pregnancy. Interestingly, the trophoblast produces most of the major pregnancy-associated hormones that have been implicated as immunomodulators, which is consistent with evidence that trophoblastic cells themselves or soluble extracts or eluates of placental tissue inhibit various expressions of cell-mediated immunity.

Other immunologic properties have been ascribed to cells derived from placenta. Trophoblastic tissue serves as an anatomic barrier between fetal and maternal tissues and thereby serves as the first line of defense against maternal antifetal alloimmunity. Maternal lymphocytes sensitized to fetal antigens could be excluded specifically, as are certain antibodies; or nonspecifically, by a generalized barrier to cellular

traffic. During early gestation, the trophoblast actively invades and proliferates within the maternal decidua, and—similar to the macrophage—it phagocytizes erythrocytes and other soluble as well as particulate material of maternal origin. Different stages of the trophoblast are highly phagocytic and capable of engulfing pathogenic microorganisms (Fig 34–1), a property that is also expressed by placental macrophages (Fig 34–2). Additional examples of placental immunocompetence include the ability of lymphoid cells from murine placentas to mediate GVH reactions, respond to mitogenic lectins, and synthesize antibodies. There is also some evidence that the placenta produces the antiviral agent interferon and the macrophage-derived immune factor interleukin-1. The expression of immunelike function by both trophoblastic elements and lymphoid stem cells may be one of several processes enabling the fetoplacental unit to protect itself from injury. This is accomplished by preventing harmful infectious agents and certain maternal antigens and antibodies from reaching the embryo and by limiting passage of cells from mother to fetus. These defense mechanisms could be especially important during the early stages of in utero development, when the fetus is quite vulnerable, since it has not yet acquired complete immune competence.

FETAL-MATERNAL EXCHANGE OF HUMORAL & CELLULAR COMPONENTS

In humans, the placenta is hemochorial, which means that there is direct apposition between the maternal circulation and the syncytiotrophoblast that lines the chorionic villi of the placenta. Although fetus and mother are grossly separated, cells as well as soluble substances can pass through the placenta during gestation, particularly at the time of placental separation (Fig 34–3).

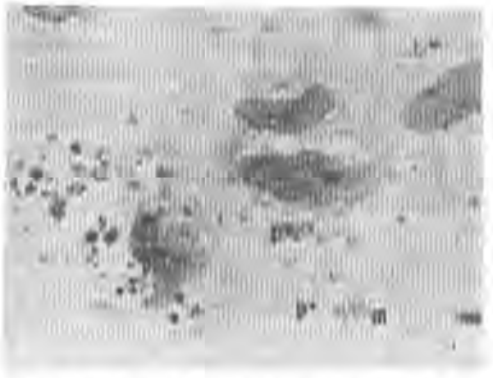

Figure 34–1. Cultured mouse ectoplacental cone trophoblast was exposed for 18 hours to the blood stage form of the rodent malaria parasite *Plasmodium berghei*. Mature intracellular forms, the schizonts (s), and extracellular merozoites (m) are readily phagocytized along with pigment granules (p). The large and irregularly shaped nuclei (N) and highly vacuolated cytoplasm of the syncytial trophoblast can also be clearly seen.

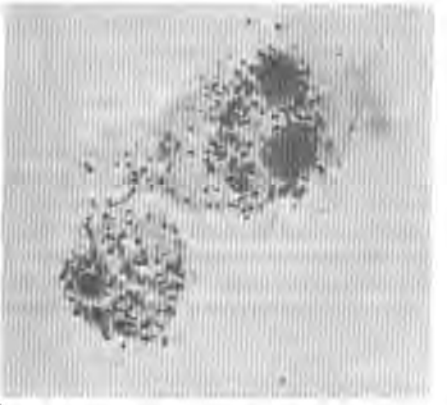

Figure 34–2. Ingestion of large numbers of rodent malaria parasites and hemozoin pigment granules by cultured mouse placental macrophages.

That such transplacental traffic results in allosensitization to transplantation antigens has been extrapolated from observations rather than proved by rigorously documented experiments. Syncytial trophoblast (more than 200,000 cells per day) is continuously released from the placenta and has been shown to circulate in human maternal blood from the 18th week of gestation. Various blood elements that undoubtedly contain transplantation antigens can pass bidirectionally. This establishes adequate conditions for sensitization of the mother by fetal (paternal) transplantation antigens. Under experimental circumstances—or clinically after intrauterine blood transfusion—immunocompetent cells that gain entrance to the fetus can rarely cause GVH disease and runting. The placenta, then, provides only a partial barrier to transport of soluble or cellular elements and certainly should not be viewed as an absolute impediment to their traffic.

In addition, whereas under normal physiologic conditions the trophoblast seems to be invulnerable to immune attack, it is quite probable that some maternal reactivity to this tissue does arise primarily to protect the pregnant host from extensive and otherwise unchecked growth and invasion of the trophoblast, as occurs in choriocarcinoma.

In the early stages of fetal development in primates, immune protection of the fetus is provided by maternally derived antibodies acquired exclusively by placental transmission. In other species, immunoglobulins are transferred via the yolk sac or via intestinal absorption of colostrum during suckling. During gestation, only antibodies belonging to the IgG class are readily transferred from mother to fetus, and this process is most likely facilitated by the interaction of immunoglobulin molecules with Fc receptors shown to be present on the surface of trophoblastic membranes and other extraembryonic membrane components.

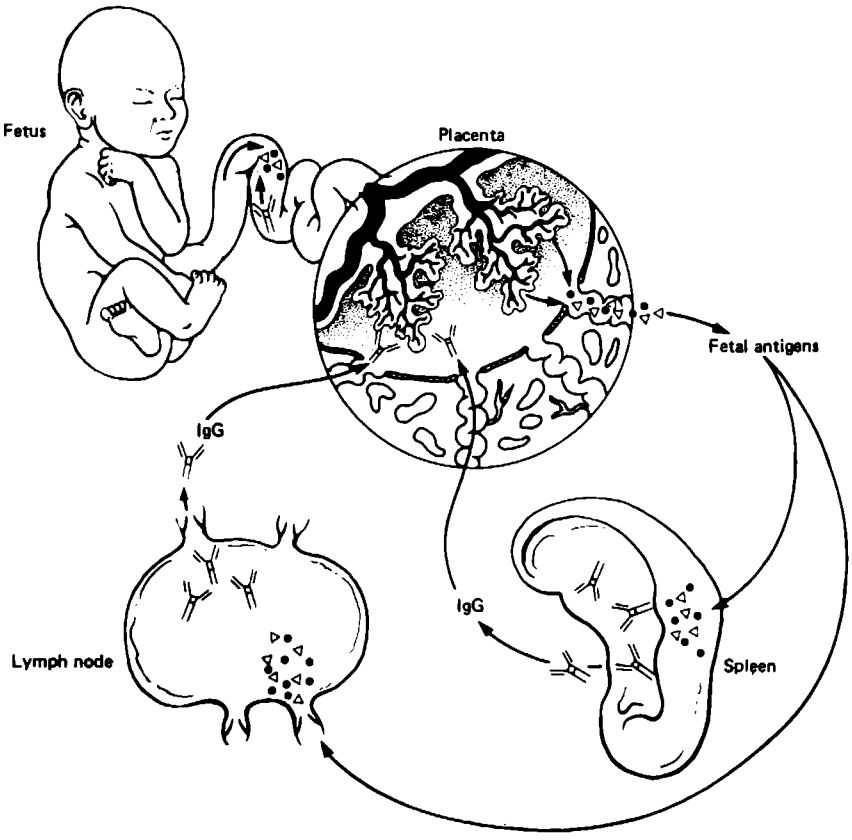

Figure 34–3. Maternal-fetal-placental complex. The anatomic features of the fetal-maternal relationship during pregnancy are depicted, with the placenta acting as a selective filter of leaking fetal or trophoblastic antigens that may sensitize maternal immune effector mechanisms. The passage of maternal immunity to the fetus is likewise regulated by the intervening layer of trophoblastic cells (where fetal and maternal tissues are in intimate proximity).

With intrauterine infection, the fetus is capable of synthesizing IgM and IgA. The presence of high levels of these immunoglobulins in cord serum at birth is presumptive evidence of such infection.

The neonate is exposed to an environment that presents much greater risk of infection than was the case in the uterine shelter. Unless in utero infection has occurred, the newborn is usually not capable of mounting a quick and effective reaction against pathogenic organisms. Maternally acquired antibody provides initial protection against infection. The presence of maternal immunoglobulins in sufficiently high titer should protect against the initial invasion of certain pathogens that would otherwise multiply and disseminate without hindrance.

MATERNAL-FETAL ANTIMICROBIAL IMMUNITY

Because of various factors related to exposure (eg, increased visits to clinics and hospitals and close association with young children), pregnant women are especially at risk of becoming infected with various types of microorganisms, including potential pathogens. The vast majority of these infections involve the upper respiratory and gastrointestinal tracts and to some extent the genitourinary tract. Occasionally, the infecting organism may invade the bloodstream and lead to fetal infection. Transplacental spread following maternal infection is the usual route by which the fetus becomes infected, although organisms can reach the fetus directly by ascending from the vagina and cervix and passing through the chorioallantoic membranes. Surprisingly, except for a few classic examples such as rubella, cytomegalovirus infection, syphilis, and toxoplasmosis, the human fetus usually remains unaffected by maternal infections. There are probably several reasons for this, including neutralization of the infectious agent by the mother through her own immune mechanisms or following antibiotic treatment; passive transfer of protective maternal antibodies to the unborn child; the presence of antimicrobial substances (lysozyme, transferrin, immunoglobulins) in the amniotic fluid; expression of resistance mechanisms (phagocytosis) in the placenta; and a limited degree of immunocompetence on the part of fetal

lymphoid tissue. For diagnostic purposes, elevated IgM in cord serum has been used as an indicator of congenital infection, since maternal IgM does not cross the placenta. This also provides further proof that the conceptus is capable of producing antibodies in response to an infection or foreign antigens.

Because of many ill effects of maternally acquired infections on fetal growth and development, many concepts derived from basic reproductive immunology are now being applied to the study of host-parasite interactions in the uterine environment. Opinion differs on whether infections occur more frequently and are more severe during pregnancy and whether this is somehow related to subtle changes or temporary dysfunction of maternal host defense mechanisms. Epidemiologic evidence indicates that pregnancy increases susceptibility to certain viral (rubella) and parasitic (malaria) diseases. However, it is not known whether this is due to increased environmental exposure to these pathogenic microorganisms, due to lack of an adequate immune response independent of or associated with various hormonal changes, or simply the result of stress during pregnancy. In contrast to patients receiving immunosuppressive drug therapy, pregnant women are not more susceptible to opportunistic infections; thus, it is highly unlikely that pregnancy causes any clinically significant or generalized weakening of the immune system. Furthermore, with an intact maternal immune system, it may even be practical to vaccinate pregnant women against certain pathogens or their toxic products. This procedure serves 2 purposes. It would provide the fetus with high-titer protective antibodies following transplacental passage, and if given to the mother in the appropriate antigenic form, enough of the antigens contained in such vaccines will reach the fetal circulation, resulting in active in utero immunization. In addition to being born with circulating antibodies synthesized de novo, the offspring would now be capable of mounting a secondary response upon subsequent exposure to the microbial antigens. Some success has already been achieved in this area. The administration of tetanus toxoid to pregnant women living in areas where tetanus is a major health problem significantly reduced the death rate due to tetanus neonatorum. It follows that other similar inactivated vaccines considered nonhazardous to the pregnant woman or her fetus, such as those presently used for influenza, typhoid fever, and diphtheria, would provide optimal protection to the offspring during the susceptible neonatal period.

IMMUNOLOGIC CONSEQUENCES OF TRANSPLACENTALLY PASSED SUBSTANCES

Under certain circumstances, transferred maternal antibody is not beneficial to the fetus. This is most evident in the classic case of hemolytic disease of the newborn mediated by either ABO or Rh blood group fetal-maternal incompatibilities. Situations involving ABO incompatibilities arise more frequently than Rh hemolytic disease but usually take a milder course, with cases requiring transfusion being extremely rare. The disorder occurs primarily in blood group O mothers bearing fetuses of type A or B and is believed to be the result of the transfer of IgG anti-A or anti-B antibodies from mother to fetus. Although blood group A and group B women have antibody to type B and type A erythrocytes, respectively, these naturally occurring isoagglutinins are usually of the IgM class and therefore do not readily cross the placenta and cannot harm the potentially susceptible fetus.

The more serious condition that can adversely affect fetal development results from Rh isoimmunization, which resembles the pathophysiology of ABO incompatibility yet manifests important immunologic differences. Rh antigens are expressed on blood cells only, whereas antigenic specificities related to A and B blood group determinants are widely distributed in nature and in the human body. During a pregnancy in which an Rh-negative mother is bearing an Rh-positive fetus, sensitization may occur if fetal red blood cells cross into the maternal circulation via the placenta or when there is transplacental hemorrhage following the birth of the child or after an abortion. Alternatively, the mother could already be primed as a result of receiving an earlier transfusion of Rh-positive blood. Less than 1 mL of fetal blood can elicit a response. Maternal antibodies pass through the placenta, gain access to the fetal bloodstream, and cause the destruction of red blood cells. Rh disease occurs rarely during the first pregnancy, with the vast majority of Rh-negative mothers becoming sensitized with increasing parity. The deleterious effects of isoimmunization can be prevented conveniently by administering Rh_0 (D) immune globulin to the mother immediately after delivery of her first Rh-positive child or following an abortion. The major suggested explanation for the action of anti-Rh immunoglobulins is that fetal erythrocytes present in the maternal circulation as a result of fetal detachment are destroyed and rapidly cleared, so that they are not available long enough to sensitize the mother effectively. Treatment must be administered after subsequent Rh-incompatible pregnancies, since there is no apparent development of tolerance following prophylactic therapy.

In certain situations, simultaneous ABO and Rh incompatibilities between mother and fetus may prevent the more destructive effects of Rh isoimmunization. The naturally occurring anti-A or anti-B antibodies in a group O, Rh-negative mother will effectively destroy Rh-positive erythrocytes crossing the placenta if the fetus is also of either group A or B blood type. The rapid removal of these cells by the already primed maternal immune system would make sensitization against Rh factor highly unlikely.

IMMUNITY & SPONTANEOUS ABORTION

Miscarriages are the most common complication of pregnancy, with the percentage of pregnancies termi-

nating prematurely being estimated at between 15% and 60%. Although certain genetic abnormalities and infectious agents have long been recognized as contributing factors, it has become apparent that underlying immune factors are responsible for the high percentage of fetal wastage. These include (1) insufficient production of important regulatory substances (inhibitors of CMI) by the gravid female; (2) the fact that couples having habitual abortions share MHC antigens at the A and B loci more frequently than would be anticipated by chance; and (3) poor distribution of the appropriate alloantigens on trophoblast tissue. These concepts are somewhat controversial and require more than the prevailing clinical and experimental evidence to make them more credible and perhaps even treatable. Nevertheless, promising clinical trials have achieved some success in reversing the rate of recurrent spontaneous abortions by immunizing abortion-prone women with paternal or allogeneic lymphocytes prior to pregnancy. Presumably, this procedure causes the wife to produce antipaternal alloantibodies that serve an important but still undefined role in protecting the developing fetus from apparent immune-mediated rejection.

REPRODUCTIVE IMMUNOLOGY IN THE MALE

Since males are not exposed to histoincompatible gametes during reproduction, immune alterations involving the male reproductive system are necessarily autoimmune in nature. Experimentally, auto- or alloimmunization to spermatozoa can in fact result in relative infertility in either the female or male. Naturally occurring autoimmune reactions to sperm are rare but have become increasingly recognized as a consequence of vasectomy. Recent findings suggest that potentially harmful immune responses associated with spermatozoa as antigens are inhibited by naturally occurring immunoregulatory substances in seminal plasma.

NATURAL IMMUNITY TO SPERMATOZOA

Sera obtained from normal, fertile animals of many species, including rabbit, mouse, and humans, have been found to contain antibodies that react with sperm of their own species and to a lesser degree of other species. In the rabbit, these naturally occurring antibodies enter the uterine secretions, probably as transudates from serum. Sperm recovered from the reproductive tract of female rabbits have immunoglobulins on the head region detectable by immunofluorescence. Interestingly, vigorously moving sperm recovered from the uterus did not fluoresce, whereas immotile sperm and those that showed evidence of senescence—as judged by alteration in the appearance of the acrosome—were bound by immunoglobulins. It has been proposed that binding of this naturally occurring antibody to spermatozoa may play a role in their clearance from the female reproductive tract, perhaps abrogating the immune response in the female to sperm-associated antigens and in maintaining a state of tolerance.

The mechanism by which antibody binding to spermatozoa occurs appears to be through Fc receptors that have been demonstrated on alcohol-fixed sperm of both rabbit and pig, which were not present on the surface of living sperm of those species. While binding of intact immunoglobulins of the IgG class was noted, Fab fragments obtained from digests of purified rabbit IgG failed to bind to sperm.

It has recently been demonstrated that an IgG preparation from serum of unimmunized rabbits mediates complement-dependent sperm immobilization. This finding suggests that a naturally occurring antibody directed against a specific sperm-associated antigen is also present within the sera of some normal fertile bucks. Cytotoxic sperm-reactive antibodies have been found in the sera of nonimmunized inbred mice. Normal serum lysed spermatozoa of all strains tested, in the presence of complement. A 3-layer indirect immunofluorescent technique using normal mouse serum, rhodamine-conjugated goat antimouse serum, and rabbit antigoat antiserum also gave a distinct surface membrane fluorescence over the whole head, principal piece, and end piece of the sperm tail from any strain when tested using living spermatozoa in suspension. Absorption of normal serum with mouse spermatozoa removed both immunofluorescent and cytotoxic activity. The cytotoxic titer of sperm-reactive antibody in normal mouse serum was low—in the range of 1:8.

Naturally occurring antisperm antibodies have been detected by indirect immunofluorescence in 90% of sera of children of both sexes before puberty. The incidence declined thereafter to about 60% and persisted throughout life. These naturally occurring antibodies were readily absorbed by sperm and testicular extracts but were not absorbed with other human tissues. The constant staining pattern with several hundred serum samples also suggested they were unlikely to be alloantibodies. Neither were they directed against blood group antigens nor sperm-coating antigens, since the antibodies were not absorbed by seminal plasma. Of special importance, these sperm-reactive naturally occurring antibodies did *not* stain the surface of viable sperm in suspension but rather were directed against intracellular antigens. Sera possessing antiacrosomal antibodies, when absorbed with lyophylized *Staphylococcus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Candida albicans,* no longer reacted with sperm by indirect immunofluorescence. In contrast, reactivity against other regions of the sperm surface was not absorbed by microorganisms.

The sera of children, female blood donors, pregnant women, and women from infertile couples have also been screened for naturally occurring sperm-reactive antibodies. Analysis of the immunofluorescent staining patterns and titers of these antibodies revealed that while 68% of children's sera were positive, only one of 84 samples tested was titered to 1:16 dilution. Similarly, for female blood donors, while 79% were positive, only 3 of 80 sera were positive at 1:16 or greater. Conversely, in women with unexplained infertility, 15 of 29 sera tested showed at least one sperm-reactive antibody at a titer 1:16 or greater. Interestingly, while 9 sera from infertile women with an immunofluorescence titer of at least 1:64 were tested by means of a microscopic sperm agglutination test, none were positive. The antibody-antigen system involved in the immunofluorescence testing appeared to be different from that detected by sperm agglutination, again suggesting that naturally occurring sperm-reactive antibodies are not directed against antigen on the sperm surface.

The sera of 2115 male partners of infertile couples have been studied by a gel agglutination test. Three percent were found to have sperm agglutinins with titers of 1:32 or greater. This low incidence of agglutinating antibodies contrasts markedly with the high incidence of antibodies reactive with sperm subsurface antigens.

Against this background of naturally occurring sperm-reactive antibodies, there has been much confusion over the role of antisperm antibodies in impaired human reproduction. The subsurface nature of Fc receptors, the mediation of immunoglobulin binding to sperm of many species via the Fc portion of the immunoglobulin molecule, and the absence of immunoglobulin binding to living human spermatozoa have not been fully appreciated. It is now clear that sera of normal fertile men and women do *not* contain antibodies directed against antigenic determinants of the sperm surface and that their presence is a reflection of an aberrant immune response that may lead—depending upon isotype, antibody specificity, and titer—to altered sperm function.

ETIOLOGY OF AUTOIMMUNITY TO SPERMATOZOA

The expression of antigens on sperm is central to the consideration of their immunogenicity in the reproductive tract, much the same as is the presence of antigens on placental trophoblast. Sperm antigens could theoretically elicit either auto- or alloimmunity, and either type could result in partial or complete infertility. Alternatively, these antigens could be nonimmunogenic in the normal environment of the reproductive tract.

The specific antigens to which antisperm antibodies are directed have yet to be defined. Newer techniques in immunoaffinity chromatography are making their identification in the near future likely. Evidence exists that antisperm antibodies in some women may be directed against antigens adsorbed to the sperm surface from seminal plasma at the time of ejaculation. Whether seminal plasma components may also be autoantigenic is unknown.

That these sperm antigens are tissue-specific rather than alloantigens is indicated by an inability to detect HLA expression on mature human spermatozoa and the finding that antisperm antibodies detected in serum of men with autoimmunity to sperm also react similarly with spermatozoa from a panel of normal fertile men. Following exposure to serum possessing sperm-reactive antibodies, unfixed frozen sections of human stomach, thyroid, ovary, and adrenal have failed to show evidence of IgG binding by immunofluorescence. The vast majority of sera from men with autoimmunity to sperm also are free of antithyroid and antinuclear antibodies as well as rheumatoid factor.

The only sperm-specific antigen well characterized at present is LDH-X, an isoenzyme of LDH mainly restricted to sperm. ABO blood group antigens are expressed on sperm only in secretors, and this important fact suggests that they are absorbed from seminal plasma. Whether these antigens are expressed in the absence of such absorption is currently moot, and no evidence for antibodies to ABO antigens in infertility has been found. The presence of Rh antigens and the haploid expression of the genes determining these blood group antigens remains controversial.

Histocompatibility antigens may be intrinsically expressed on sperm and not absorbed from seminal plasma. Ia antigens in the mouse are clearly detectable on sperm from epididymis and ductus deferentes, the regions of the male reproductive tract where seminal plasma has not yet been formed. Whether both parental haplotypes are expressed in sperm remains unresolved. The presence of HLA antigens on sperm is controversial. Recently, studies with monoclonal antibodies directed at framework determinants of HLA-A, -B, -C, and -D antigens and employing very sensitive radioimmunoassays and immunofluorescence assays failed to find any HLA antigens in epididymal or ejaculated sperm. Evidence for the presence of HLA-D antigens, the probable human analogs of murine Ia antigens, has been derived primarily from the ability of sperm to stimulate lymphocytes in allogeneic mixed sperm lymphocyte cultures. Conclusions from these experiments have been criticized by some because the sperm stimulator cell population was contaminated with small numbers of other cells from the reproductive tract, especially leukocytes known to express HLA-D antigens. Serologic methods for HLA antigens that do not employ monoclonal reagents may harbor non-HLA antibodies, thereby giving false-positive results. A definitive resolution to this very important issue of HLA expression on sperm awaits further studies with these specific reagents.

H-Y antigen, which is encoded as a male-specific antigen by a gene on the Y chromosome and is central to primary sex differentiation, appears to be expressed

on sperm. If sperm expressed exclusively either Y- or X-determined antigens, sex determination by antibodies directed at either X- or Y-bearing sperm would theoretically be possible. However, most investigators have not been able to show haploid expression of genes determining H-Y or H-X antigens on sperm. Thus, the possibility of exploiting haploid gene expression for sex determination appears remote.

Unresolved questions in the important area of sperm antigens include (1) whether transplantation antigens are expressed in a haploid or diploid mode, (2) the identity of a sperm-specific antigen, and (3) the definition of antigens that give rise to antibodies which cause infertility in males or females.

A high incidence of sperm-reactive antibodies has been detected in the sera of homosexual men. Oral sex or deposition of sperm within the rectum during intercourse may result in immunization to sperm antigens. The distribution of immunoglobulin isotypes of sperm-reactive antibodies in the sera of these homosexual men is quite different from that seen in heterosexual men from infertile couples. While tail-directed antibody of the IgG class—and, to a lesser extent, IgA—predominate in the latter group, head-directed IgMs are found more frequently in the sera of homosexuals. The nature of the antigens to which these antibodies are directed is unknown.

It has been suggested that genital tract infections may lead to the development of autoimmunity to sperm. However, such individuals may constitute only a small proportion of the total population of men in whom sperm-reactive antibodies are detected. In a study of 324 consecutive ejaculates submitted for semen analysis and sperm antibody studies, 46 were found to possess autoantibodies to sperm. The incidence of pyospermia (greater than 1 million PMNs per milliliter), as judged by acridine orange staining and fluorescence microscopy, was comparable in the 2 groups at 10.8% and 15.2% of those antibody-negative versus antibody-positive. Four of 46 ejaculates of autoimmune men were found to have more than 5 million PMNs per milliliter, versus 9 of 278 ejaculates that were antibody-negative. Although this observation does not preclude a prior acute infection, it suggests that chronic genital tract infection is an unlikely cause of autoimmunity to sperm. Most men studied also denied knowledge or symptoms of acute prostatitis or epididymitis.

A large number of lymphocytes, ranging from 42,000 to 29 million, have been identified in the semen of normal heterosexual men. Recently, using monoclonal T cell probes and immunoperoxidase staining, a population of intraepithelial CD8-positive suppressor T lymphocytes has been identified within the human epididymis. It has been postulated that these cells might play a role in preventing the development of autoimmunity to spermatozoa by acting locally to limit B cell differentiation and production of specific antibody against sperm-associated antigens. Alternatively, these T cells might suppress antigen processing and presentation by macrophages.

METHODS OF DETECTING SPERM-REACTIVE ANTIBODIES

Many techniques have been described over the years for detection of sperm-reactive antibodies in serum and other body fluids. It has become increasingly apparent that there are 2 sources of immunoglobulins within the male genital tract. These include those present as transudates from serum and those locally secreted. Therefore, circulating sperm-reactive antibodies may not be representative of those antisperm antibodies present in semen. Infertile couples were tested for the presence of antisperm antibodies, either when impaired sperm cervical mucus-penetrating ability was noted on postcoital testing despite normal semen analysis or in the face of idiopathic infertility. During a 5-year period, 1825 semen specimens were submitted to the Laboratory of Human Reproduction at North Shore University Hospital, Manhasset, NY. Sperm were washed free of seminal fluid by low-speed centrifugation and tested directly by immunobead binding for the presence of surface-bound immunoglobulins. Humoral antisperm antibodies were detected by incubation of known antibody-free spermatozoa (husband or donor) in dilute patient serum (1:4), following which sperm were washed free of serum and tested by immunobead binding. Autoantibodies were detected on the sperm surfaces in 13% of ejaculates. In 846 men, matched serum and semen specimens were studied, and comparison could be made of humoral antibodies with those present within the genital tract. Twenty percent of men were found to have sperm-reactive antibodies in their blood without any detected on spermatozoa.

Although humoral antibodies may enter the seminal plasma as transudates, total immunoglobulin levels in semen are about one-tenth lower than those seen in serum. Male infertility has previously been noted in association with high titers of circulating antisperm antibodies. Indeed, in cases where sperm-reactive antibodies were present in blood but not detected within the ejaculate, serum immunobead binding levels were low. Bearing in mind that immune phenomena wax and wane and that these men with low-grade autoimmunity to sperm require further surveillance, it would seem that the absence of antisperm antibodies in the ejaculate means that they do not provide an immune basis for infertility.

Conversely 15% of men were found to have antibodies present on the sperm surface, but none were detected in serum. These antibodies are primarily of the IgA class, although IgGs may also be detected. The predominance of IgAs in semen—and their absence in serum—suggests their local production within the genital tract. Whether these autoantibodies originate in the accessory gland secretions (and are encountered by sperm at the time of ejaculation) or within the epididymis is unknown. In the rabbit, the epididymis is capable of local secretion of antibodies.

In combination, one-third of serologic tests performed to detect antisperm antibodies provided mis-

leading information that could lead to an error in clinical management. These results emphasize the need to study the ejaculate directly rather than solely using serologic tests in the diagnosis of immune-mediated male infertility.

DETECTION OF SPERM-ASSOCIATED IMMUNOGLOBULINS

Several methods are now clinically available to determine whether spermatozoa themselves are immunoglobulin-bound. These include the mixed agglutination reaction, a direct antiglobulin assay using ^{125}I-radiolabeled heterologous antibody, direct ELISA, and immunobead binding. Although each of these tests allows one to determine, in a semiquantitative way, the extent of autoimmunity to sperm, immunobead binding in particular provides a measure of the proportion of spermatozoa in the ejaculate antibody bound by each of the 3 major immunoglobulin isotypes (IgG, IgA, and IgM). The precise amount of immunoglobulin associated with the individual spermatozoal surface, however, still cannot be determined by current methods.

DETECTION OF HUMORAL ANTIBODIES

Sperm Immobilization Tests

Antibodies absolutely dependent on complement to produce immobilization of sperm were initially described by Fjällbrant in 1965 and Isojima in 1968. In these assays, donor sperm from the male to be tested—or from a normal control—are washed and incubated with serial dilutions of heat-inactivated test serum or other secretions. These can be derived from the male to be tested or from female partners. A source of complement (usually fresh guinea pig serum) is added. A time end point for immobilization (Fjällbrant) of 90% sperm—or a percentage of motile sperm at a standard time (Isojima)—is compared microscopically with sperm incubated in control sera and complement alone. Complement-dependent immobilization, while highly specific in that false-positive reactions are uncommon, will not detect the presence of non-complement-fixing immunoglobulins. The degree of antibody present on the sperm surface also appears to play a role in the extent of complement-dependent immobilization. Results obtained from immobilization tests have a definite relationship to immunologic infertility. However, correlation of sperm-immobilizing antibodies with agglutinating antibodies is not perfect.

Since seminal plasma contains complement inhibitors, complement-dependent cytotoxicity tests cannot be applied to detection of autoantibodies to sperm in semen. Although sperm agglutination, antiglobulin tests, radiolabeled protein A, and ELISA have all been applied to the study of antisperm antibodies within the seminal plasma, they suffer from the fact that the majority of sperm-reactive antibodies present within the ejaculate may be cell-associated (sperm-bound) rather than cell-free. If antibody concentrations are limiting relative to the number of antigenic sites on the sperm surface, there may be no residual antibody within the seminal plasma despite its presence bound to spermatozoa. Those antibodies detected within seminal plasma may then not be reflective of immunoglobulins associated with sperm.

Sperm Agglutination Tests

Agglutination tests are a sensitive and specific means of detecting sperm antibodies. The 2 main procedures for sperm agglutination are the gelatin agglutination test of Kibrick and the microagglutination test read macroscopically. In the Franklin-Dukes method, no gelatin is used, and agglutination is read on slides or in a microtiter tray in the microscope. Agglutination may be primarily head-to-head or tail-to-tail, rarely head-to-tail. Tail-to-tail agglutination usually occurs in female sera and head-to-head in male sera. The antigens detected by this test are not fully characterized.

Failure to give careful attention to controls in sperm antibody tests has often led to misleading results. Obviously, obtaining a standard source of viable human sperm presents difficulties. Known positive and negative control sera must be included.

Other Antibody Tests

In response to the need for assays that correlate better with clinical evidence of infertility, a variety of new tests have appeared.

While a number of enzyme-linked immunosorbent assays have been developed to detect the presence of sperm-reactive antibodies in serum or semen, there has recently been increasing dissatisfaction with this approach. A high incidence of "naturally occurring" sperm-reactive antibodies in the sera of fertile men and women of all ages poses a major problem of "immunologic background noise" for the ELISA. These antibodies, which react with subsurface components of spermatozoa, are not expected to interfere with the membrane-associated interaction of gametes that lead to successful fertilization The method of fixing spermatozoa is critical in determining which antigens are "presented" to a test serum sample. In particular, when spermatozoa are air-dried, disruption of the sperm plasma membrane can provide access to internal antigens. A marked variation in the ability of an ELISA to detect sperm-reactive antibodies has been documented when spermatozoa were fixed in different manners, eg, air-drying to wells, glutaraldehyde fixation, reacting with test serum when living, or following freeze-thaw.

Another approach has been to utilize extracts of spermatozoa as the target antigens for ELISA. It has been hypothesized that lithium diiodosalicylate extracts those sperm membrane-associated antigens that are relevant to infertility. However, they may be absent or altered beyond recognition by antibody. When several laboratories, each utilizing its own ELISA methodologies, studied a group of clinically defined

serum provided by the WHO Reference Bank, there was no uniformity of results between groups. Different ELISAs thus appear to detect different groups of sperm-reactive antibodies, and which of these antibodies are relevant to impaired reproduction remains unknown.

Ideally, those sperm-reactive antibodies detected by a particular laboratory method must be shown by either clinical studies or by in vitro gamete interaction to alter sperm function. A correlation has been found between the proportion of spermatozoa that bind immunobeads in ejaculates of men with autoimmunity to sperm and the number of spermatozoa seen within cervical mucus on postcoital testing. Sperm penetration into cervical mucus was nearly absent despite the presence of normal numbers of motile sperm in the ejaculate, when more than 80% of sperm were antibody-bound. Conversely, increasing numbers of spermatozoa were noted at postcoital testing, as immunobead binding levels dropped under 50%. The extent of autoimmunity to sperm, as reflected in the proportion of sperm-binding immunobeads, also correlated with the chance that pregnancy would occur in couples when female causes of infertility had been addressed but no specific treatment has been offered the husband.

Mathur has introduced a new sensitive and specific assay for cytotoxic antisperm antibodies. This double immunofluorescence test depends on diacetyl fluorescein, which stains viable sperm, and on ethidium bromide, which counterstains dead sperm. In the presence of complement, this assay can detect both auto- and isoantibodies to sperm in infertile couples. Results of the sperm cytotoxicity and passive hemagglutination studies were compared with those obtained by immunobead binding, tray-agglutination and gel-agglutination, in a group of clinically defined sera provided by the WHO Reference Bank. There was no correlation between assays, which suggests that a different group of antibodies was being detected by the former procedures. Emphasis must be placed on whether the results of particular methodologies correlate with impaired sperm function, either clinically or in the laboratory.

ROLE OF ANTISPERM ANTIBODIES IN INFERTILITY

Antisperm Immunity in the Male

There is little doubt that antibodies directed toward various sperm antigens can result in reduced fertility in men. Results from several large series on the presence of sperm agglutinins in fertile and infertile men place the cutoff point for a significant titer of sperm-agglutinating antibodies at approximately 1:32. In most such studies, the correlation between sperm agglutinins and complement-dependent immobilizing antibodies is reasonably high. However, the tendency is toward lower titers and fewer fertile individuals with positive immobilizing antibodies. Thus, immobilizing antibodies are mainly specific. Although agglutinating antibodies are a sensitive index for infertility, their specificity is poor.

Evidence exists that sperm-reactive antibodies may impair sperm function. Sperm entrance into cervical mucus from seminal plasma is impaired in the presence of auto-antibodies to sperm. It can be shown both clinically, at postcoital testing, and in vitro. Spermatozoa from known fertile donors previously shown to be able to penetrate human cervical mucus failed to do so following incubation with antibody in vitro. The Fc portion of the immunoglobulin has been implicated, in restricting sperm motion within cervical mucus, since donor spermatozoa bound to Fab fragments of sperm-reactive antibodies show no impairment of sperm penetration within cervical mucus.

Spermatozoa bound by immunoglobulins at the acrosomal and postacrosomal regions of the sperm head may be impaired in their ability to fertilize eggs, even if they reach the site of fertilization within the distal ampulla of the uterine tube. Species-specific receptors for the zona pellucida have been identified on the plasma membrane of sperm. Antibodies raised experimentally against boar sperm plasma membranes block sperm attachment to porcine zonas. Spontaneously occurring autoantibodies in men from infertile couples directed against surface antigens of the sperm head have also been shown to impair the ability of human sperm to attach to the zona pellucida of nonliving human ova.

Several laboratories have now reported the ability of antisperm alloantibodies in women—as well as autoantibodies in men—to impair penetration of zona-free hamster ova by human sperm. The penetrating ability of spermatozoa was diminished markedly in the presence of sperm-reactive antibodies fixed to complement but not when IgA non-complement-fixing sperm-reactive antibodies were present.

Most agglutinating antibodies to sperm in seminal plasma are of the IgA class, and immobilizing antibodies are predominantly IgG. Agglutinating IgA antibodies may exist only in seminal plasma. IgM only rarely gains access to seminal plasma with a significant inflammatory lesion in the reproductive tract or after vasectomy.

Other Consequences of Autoimmunity to Sperm in the Male

Despite the presence of high levels of autoantibodies to sperm within the reproductive tract, sperm output is not impaired, and the distribution of sperm concentrations within semen is similar to that seen in infertile men in the absence of autoimmunity to sperm.

A blood-testis barrier exists as tight junctional complexes between Sertoli cells and divides the seminiferous tubule into basal and adluminal compartments. In the dark mink, naturally occurring orchitis is associated with breakdown of tight junctions between Sertoli cells, suggesting that the blood-testis barrier might become defective during seasonal regression of the testis. Experimental immunization of guinea pigs with testis extracts containing specific autoantigens

has also been associated with the development of immune orchitis. Specific plasma membrane antigens may be present on both mature epididymal spermatozoa and earlier stages of sperm development within the testis. In humans, however, there is no evidence that autoimmunity to sperm—either occurring spontaneously in infertile couples or following vasectomy—is associated with the development of clinical orchitis. In a single study, a man with prostatic cancer destined to undergo orchiectomy was immunized with spermatozoa and the testis subsequently examined for evidence of orchitis. Only focal lesions were noted, suggesting that those antigens present on mature spermatozoa may not be expressed during development of precursor sperm within the testis. Unfortunately, the cancer itself may have altered this individual's immune responsiveness to sperm antigens. This may vary between species, as suggested by the propensity of certain animals to develop an experimental allergic orchitis. The failure of vasectomy to be associated with the development of clinical orchitis in humans also suggests a different pattern in the stage-specific expression of antigens on primate sperm.

In a further attempt to document whether spontaneously occurring sperm-reactive antibodies in the sera of men from infertile couples might cross-react with intratesticular spermatozoa, 3.5-μm frozen, unfixed sections of human testis were exposed to 13 sera that possessed sperm-reactive antibodies and examined by indirect immunofluorescence. There was no evidence of binding of human IgG to spermatozoa or spermatocytes within the seminiferous tubules. This result suggests that antibodies directed against antigens present on the surface of ejaculate sperm did not make their appeareance until after the completion of spermatogenesis. Alternatively, loss of relevant antigens might have occurred following orchiectomy or in preparation of the tissues for study.

Several studies using in vitro correlates of cellular immunity to sperm in infertile individuals have been performed. These have for the most part given conflicting results. Neither lymphocyte transformation nor leukocyte inhibitory factor production has given consistent evidence for cellular sensitization in individuals with sperm immunity. Clearly, this is an important area requiring additional study.

Various forms of therapy have been employed to reduce antibodies to sperm in the male. These include systemic administration of adrenal corticosteroids, attempts at artificial insemination with washed sperm, and, in the case of antibodies in the female, condoms to reduce antibody titers by reducing exposure to sperm. The success of all of these forms of treatment is limited. Further studies are needed to fully confirm their efficacy, especially in controlled clinical trials.

IMMUNOLOGIC CONSEQUENCES OF VASECTOMY

About a million vasectomies are performed annually in the USA. As a result of this procedure, antibodies and probably cellular immunity to sperm develop in most men as a result of interaction of extravasated sperm antigens with the immune system. Sperm are autoimmunogenic, mainly because of their normal sequestration behind a blood-testis barrier and their late development relative to the establishment of self tolerance. The potential adverse consequences of this immune response to sperm include (1) systemic effects on other organ systems and (2) interference with fertility after reanastomosis of the vasa deferentia (vasovasostomy).

There is no doubt that vasectomy in humans leads to production of sperm antibodies. Most studies indicate a 60–70% incidence of sperm agglutinins in serum and a 30–40% incidence of immobilizing antibodies by 1 year following the procedure that persist for as long as 10 years in about half of the initially positive patients.

Granuloma formation due to local extravasation of sperm is relatively common, but the presence of granulomas correlates poorly with antibody to sperm. The antibodies that develop after vasectomy are tissue-specific and do not react with HLA or Ia antigens. A recent study showed a strong association of HLA-A28 with production of sperm antibodies following vasectomy, suggesting a genetic predisposition. Normal semen contains little or no IgM, but its presence following vasectomy and with antibodies for sperm relates to the degree of chronic inflammation from extravasal spermatozoa.

Little is known about cellular immunity to sperm following vasectomy. Several studies in animals immunized with sperm or undergoing vasectomy have shown inconstant development of T lymphocyte responsiveness to sperm. In vitro cellular immune studies in humans are still inconclusive. The well-recognized in vitro immunoinhibitory action of seminal plasma may in fact prevent such responses in vivo. Much more needs to be learned about other components of the immune response to sperm following vasectomy.

There are no established adverse systemic immune effects of vasectomy in humans. In 1968, a few patients with vasectomy were reported with either thrombophlebitis, glomerulonephritis, or multiple sclerosis, but these sequelae have not been observed in larger series. Sex hormone levels are not influenced by vasectomy. A slight increase in antinuclear and anti-smooth muscle antibodies has been demonstrated in a few individuals. There is evidence for circulating immune complexes composed of sperm antigens and antibody following vasectomy in men.

Vasectomy can accelerate atherosclerosis in monkeys fed atherogenic diets. The postulated mechanism involves deposition of sperm-antisperm immune complexes in vascular intima followed by plaque formation. A large epidemiologic study in the USA using case controls (1512 subjects) or cohort analysis (1764 men) revealed no evidence for increased cardiovascular disease in a 6- to 7-year follow-up period. This study has been confirmed by Danish workers as well.

Nevertheless, continued immunologic and clinical monitoring of vasectomized men seems warranted.

A surgical method for reanastomosis of ligated vasa deferentia, termed vasovasostomy, has been successfully developed. The presence of sperm antibodies in 60–70% of vasectomized men represents a potential threat to restoration of full fertility. There are conflicting studies about whether the presence of sperm antibodies results in decreased fertility in such individuals. However, the well-documented antifertility effects of high titers of sperm antibody make it likely that fertility may not be fully restored.

IMMUNOLOGIC FEATURES OF SEMINAL PLASMA

Seminal plasma contains a variety of potentially antigenic substances, particularly enzymes, on enzymatic proteins and nonproteinaceous substances. Immunoglobulins are also present but at much lower concentrations than in serum (IgG, 7–13 mg/dL; IgA, 2–6 mg/dL). IgM is not normally detectable. Other substances with potential regulatory effects in the immune system include transferrin, zinc, prostaglandins, and polyamines.

Several potentially important immunoinhibitory substances have been detected in seminal plasma. One of these substances has broad-spectrum immunosuppressive effects on lymphocyte function in vitro, including blocking proliferation of T cells stimulated by mitogen, antigen, and allogeneic cells. They also block in vitro T cell-dependent or -independent antibody products of B cells. Although this factor is apparently a macromolecule present in several species, direct evidence of an in vivo immunosuppressive effect is lacking.

Seminal plasma interferes with a variety of microbicidal functions. Bactericidal and opsonic activity of serum or granulocytes was blocked for a variety of microorganisms, including *Neisseria gonorrhoeae* and *Escherichia coli* but not *Staphylococcus aureus*. The precise mechanism by which this inhibitory activity is mediated has not yet been determined. Complement activation is reduced by incubation with seminal plasma, which contains a large variety of proteases and protease inhibitors. It is interesting to speculate that the various immunoinhibitors in seminal plasma have a role in protecting sperm from immunologic attack in the female reproductive tract. In pathologic states, infectious agents may escape destruction in the reproductive tract as a consequence of inhibition of microbicidal action.

Anaphylaxis due to components of semen (either sperm or seminal plasma) has been rarely described. Symptoms occur immediately following sexual intercourse and include urticaria, angioedema, and occasionally hypotension. Immediate hypersensitivity to intrinsic seminal plasma antigens has been demonstrated. Therapy consists of either abstinence or use of a condom to prevent contact with semen and has proved somewhat successful. A patient with severe allergy to seminal plasma underwent normal pregnancy and delivery after artificial insemination with seminal plasma-free spermatozoa.

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35 Parasitic Diseases

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Parasitic diseases such as malaria, schistosomiasis, and leishmaniasis are among the most important health problems in developing countries. Because vector control and other public health measures have failed to eradicate these diseases, WHO/UNDP/World Bank are undertaking a long-term Special Programme for Research and Training in Tropical Diseases in which development of immunization procedures plays an important role. Increased travel to all parts of the globe now makes the solution to the problem of parasitic disease imperative.

Not only is understanding of the immunology of parasitic disease essential in order to control these diseases by immunization—the study of the host response to parasites continues to lead to important discoveries about the immune response itself. For example, the response to schistosome eggs by infected mice represents one of the best experimental models for studying the formation and regulation of granulomatous inflammation. Immature schistosomes (schistosomula) and eggs have also provided an in vitro experimental model for elucidating the function of the eosinophil.

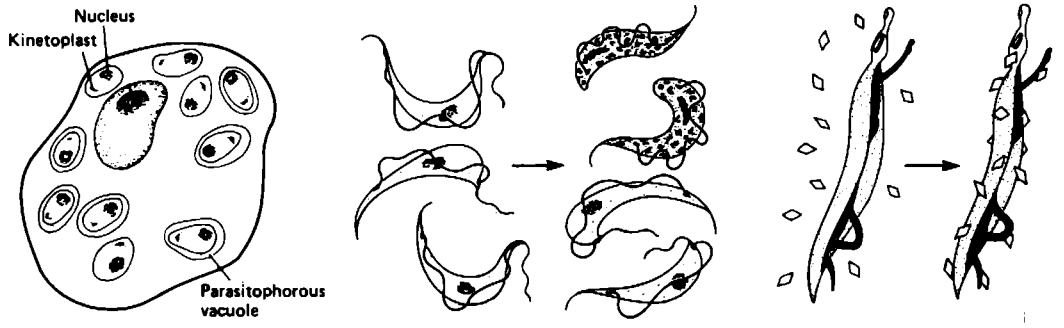
Immune responses to the complex antigenic structures of parasites have diverse manifestations. For example, immunity with specific protection to reinfection occurs after primary infection with cutaneous leishmaniasis; in falciparum malaria, partial protection against recurrent infection results from persistently low levels of parasitemia, which stimulates production of protective antibody (concomitant immunity or premunition).

Unfortunately, as is the case with other infectious diseases also, the immune response to parasites can produce more serious disease than the parasite itself. Examples are the hepatic granulomas of schistosomiasis, antigen-antibody complex glomerulonephritis in quartan malaria, and antibody-mediated anaphylactic shock from a ruptured hydatid cyst or from too-rapid killing of filarial microfilariae.

Some of the most fascinating and perplexing aspects of parasitic disease are the variety of mechanisms by which the parasite evades the immune response (Fig 35–1). A parasite can "hide" within a host's own cells, as in leishmaniasis; disguise itself as "self" with host antigens, as in schistosomiasis; or produce successive waves of progeny with different surface antigens, as in African trypanosomiasis. Nonspecific immunosuppression, due to a variety of stimuli, is characteristic of a number of parasitic infections. The ability of parasites to adapt to the host environment is the essence of successful parasitism, and it increases immeasurably the difficulty of developing immunization procedures against parasitic infection.

Because the host serves as an in vivo medium for parasite survival and multiplication, its state of health, age, nutrition, emotional state, and concurrent infection are all significant factors in pathogenesis of parasitic disease.

New interest and research by immunologists,



A. Living within a host cell (*Leishmania* living in macrophage within a parasitophorous vacuole)

B. Rapidly changing surface antigens (trypanosomes)

C. "Camouflaging" surface with host antigens

Figure 35–1. Some of the devious ways in which parasites evade the host immune response.

molecular biologists, and parasitologists promise significant advances in parasite immunology within the next few years. Efforts are under way to develop prophylactic or therapeutic vaccines against malaria and schistosomiasis. Serodiagnostic techniques have also undergone significant recent improvement in specificity and sensitivity. Even better reagents may shortly be available for serodiagnosis of a number of parasitic diseases as specific purified antigens are amplified using recombinant DNA technology. Available serodiagnostic procedures are listed in Table 35–1 and the routine tests done at the Centers for Disease Control in Table 35–2.

THE IMMUNE RESPONSE TO PROTOZOA

Protozoa are important agents of worldwide disease. Falciparum malaria, for example, is still one of the most lethal diseases in humans in spite of massive efforts at eradication and control. These parasites also offer unlimited immunologic challenges, since the immune responses they induce are as diverse as the protozoa themselves. In developing countries, especially in Africa, malaria and trypanosomiasis take enormous tolls of life and are significant barriers to economic development, survival of domestic animals, and human occupation of vast grazing lands. Amebiasis, giardiasis, and toxoplasmosis are widespread even in highly developed countries. The use of immunosuppressive drugs to treat cancer and to prevent rejection of transplanted organs has resulted in activation of otherwise subclinical infections with protozoa such as *Toxoplasma* and *Pneumocystis* or has induced an overwhelming systemic infection with the nematode *Strongyloides stercoralis*. In some instances, deaths have been caused by these infections rather than by the underlying illness for which treatment was being given. *Pneumocystis* pneumonia is the most common fatal complication of acquired immunodeficiency syndrome (AIDS).

MALARIA

Major Immunologic Features

■ Species-specific protective IgG antibody pro-

Table 35–1. Immunodiagnostic tests for parasitic diseases.*

Parasitic Diseases \ Tests	Complement Fixation	Agglutination Tests: Bentonite Flocculation	Agglutination Tests: Indirect Hemagglutination	Agglutination Tests: Latex	Agglutination Tests: Special Agglutination	Indirect Immunofluorescence	Immunodiffusion	Immunoelectrophoresis	Countercurrent Electrophoresis	ELISA	Intradermal
Amebiasis	■	▲	■	▲	○[A]	■	■	■	▲	○	▲
Chagas' disease	■		■	▲	▲[A]	■	○	▲	○	▲	○
African trypanosomiasis	▲		○		○[C]	▲	▲			○	
Visceral leishmaniasis	■		■	○	▲[A]	▲	○	○			■
Malaria	▲		■	▲		■	■	○	○	○	
Pneumocystosis	■			○		■					
Toxoplasmosis	■		■	○		■	○			○	■
Ancylostomiasis	○		■	▲		○		○			▲
Ascariasis	○	■	■			▲	▲	○		○	▲
Clonorchiasis	■		■					○			■
Cysticercosis	▲		▲	▲		▲	▲	○	○		○
Echinococcosis	■	■	■	▲		■	▲	■	○	○	■
Fascioliasis	■	○	■	▲		▲	○	○	○		▲
Filariasis	▲	■	■	○		■		○			▲
Paragonimiasis	■	○	○				○	○			■
Strongyloidiasis			■			▲					
Schistosomiasis	■	▲	■	○	■[C]	■	▲	○		○	■
Toxocariasis	○	■	■			▲	○	○		○	▲
Trichinellosis	■	■	■	■	■[B]	■	▲	▲	○	▲	■

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Legend:

■ = Evaluated test ▲ = Experimental test ○ = Reported in the literature

Special agglutination tests:

A = Direct agglutination B = Cholesterol agglutination C = Charcoal agglutination

Table 35–2. Serologic tests for the diagnosis of parasitic diseases performed in the Parasitology Division of the Centers for Disease Control.*

Diseases	Tests	Diagnostic Titers
Amebiasis	IHA	≥1:128
Chagas' disease	CF, DA, IHA	≥1:32, ≥1:128, ≥1:128
Leishmaniasis	IHA, DA	≥1:64, ≥1:64
Malaria	IIF	≥1:64
Pneumocystosis	DIF	≥1:16
Toxoplasmosis	DIF, (IgM-IIF), IHA	≥1:256, ≥1:16, ≥1:128
Ascariasis	IHA, BF	≥1:128, ≥1:5
Filariasis	IHA, BF	≥1:128, ≥1:5
Toxocariasis	IHA, BF	≥1:128, ≥1:5
Strongyloidiasis	IHA	≥1:64
Trichinellosis	BFT	≥1:5
Paragonimiasis	CF	≥1:16
Schistosomiasis	CF, IIF	≥1:8, ≥1:16
Cysticercosis	IHA, BF	≥1:128, ≥1:5
Echinococcosis	IHA, BF	≥1:128, ≥1:5

IHA = indirect hemagglutination; CF = complement fixation; DA = direct agglutination; DIF = direct immunofluorescence; IIF = indirect immunofluorescence; BF = bentonite flocculation.

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duced against merozoites after multiple infections.

- Immunity to sporozoites can be induced using tandemly repeated peptides from the parasite's surface.
- High serum immunoglobulin levels in endemic areas due only partly to malaria itself.
- Immunosuppression of other antibodies during course of disease.
- Parasites display antigenic variation.
- Great variety of humoral antibodies elicited as well as increased reticuloendothelial activity.
- Complex state of partial immunity induced after many years and multiple exposures, involving humoral and cellular, specific and nonspecific, hereditary and acquired characteristics—as well as the effect of a current low-level infection on a challenge infection (premunition).

Human malaria is caused by species of *Plasmodium*. It is transmitted by female anopheline mosquitoes that ingest the sexual forms of the parasite in blood meals. The infective sporozoites develop in the mosquito and are injected into the definitive (human) host when bitten by the insect. In the human, the parasites first develop in an exoerythrocytic form, multiplying within hepatic cells without inducing an inflammatory reaction. The progeny, or merozoites, invade host erythrocytes to begin the erythrocytic cycle and initiate the earliest phase of clinical malaria. Destruction of red cells occurs on a 48-hour cycle with *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium falciparum* and every 72 hours with *Plasmodium malariae*. The characteristic chills-fever-sweat malarial syndrome follows this cyclic pattern, being induced by synchronous rupture of infected red cells by the mature asexual forms (schizonts), releasing merozoites that quickly invade new red cells. In contrast to the exoerythrocytic stage, these erythrocytic merozoites induce an array of humoral responses in the host, as demonstrated by complement fixation, precipitation, agglutination, and fluorescent antibody reactions.

Relapse after a period of dormancy results from periodic release of infective merozoites from the liver, which lacks an immune response to the intracellular parasites. This is apparently determined by the genetic constitution of the invading parasite sporozoites. When the erythrocytic cellular and humoral protection is deficient (from concurrent infection, age, trauma, or other debilitating factors), the reappearing blood-stage forms induce a new round of clinical malaria until the erythrocytic cycle is again controlled by a humoral and thymus-dependent cell-mediated host response. True relapse, as opposed to a delayed exoerythrocytic cycle or a recrudescence of erythrocytic infection, generally will occur for up to 5 years with some strains of *P vivax* and possibly 2–3 years for *P ovale*. *P malariae* appears to recur only as a recrudescent erythrocytic infection, sometimes lasting 30 or more years after the primary infection. *P falciparum* may have a short-term recrudescence but does not develop a true relapse from liver-developed merozoites.

Blackwater fever, formerly a common and rapidly fatal form of falciparum malaria among colonists in Africa, has declined in frequency with reduction in quinine therapy. It is associated with repeated falciparum infection, inadequate quinine therapy, and possibly genetic factors more frequently found in whites. The resulting rapid, massive hemolysis of both infected and uninfected red cells is thought to result from autoantibodies from previous infections that react with autoantigens (perhaps a red cell-parasite-quinine combination) derived from a new infection with the same falciparum strain. With increased use of quinine to

prevent or treat chloroquine-resistant falciparum malaria, blackwater fever may increase in frequency in coming years.

Quartan malaria, caused by *P malariae*, in African children has been associated with a serious complement-dependent antigen-antibody immune complex glomerulonephritis and nephrosis, resulting in edema and severe kidney damage unless the disease is arrested early. After loss of the edema, persistent symptomless proteinuria or slowly deteriorating renal function is common. Stable remission with corticosteroid therapy occurs when proteinuria is restricted to only a few classes of protein and histologic changes are minimal. But patients with poorly controlled generalized proteinuria are probably not benefited by antimalarial or immunosuppressive therapy. Chronic *P malariae* infection probably triggers an autoimmune mechanism perpetuating the immune complex glomerulonephritis, but the antigen involved is not yet identified.

Innate, nonacquired immunity to malaria is well demonstrated. African or American blacks lacking Duffy blood group antigen Fy(a-b-) are immune to *P vivax*, as this genetic factor appears to be necessary for successful merozoite penetration of the human red cell by this plasmodial species. Intracellular growth of the malaria parasites is also affected by the hemoglobin molecular structure. Sickle cell (SS) hemoglobin inhibits growth of *P falciparum*. This genetic factor is widespread in areas of Africa hyperendemic for falciparum malaria. Though prevalence of infection appears unaffected by the sickling trait, *severe* infections in individuals with hemoglobin A/S (sickle cell trait) are very much reduced compared with those in nonsickling homozygote individuals. Similarly, *P falciparum* growth is retarded in red cells with the fetal hemoglobin (F)—hence the selective advantage of β-thalassemia heterozygotes, in whom postnatal hemoglobin F declines at a lower than normal rate. Other red cell abnormalities such as glucose-6-phosphate dehydrogenase deficiency appear on epidemiologic grounds to be protective of the red cell and reduce the severity of plasmodial infection.

Both acquired and innate specific or nonspecific resistance to malaria is influenced by a number of genetic traits that reflect strong selective pressure in areas with specific mosquito-human-*Plasmodium* combinations. Though we have much to learn about expressions and mechanisms of malaria resistance from rodent and monkey malaria studies, direct application to humans or broad generalizations about acquired or natural immunity cannot be readily drawn from these host-parasite experiments. A very gradual long-term resistance to hyperendemic falciparum malaria is acquired in African populations. The resistance develops years after the onset of severe disease among nearly all children over 3 months of age. Initial passive protection is present owing to transplacental maternal IgG. There are estimates of a million malaria deaths a year in Africa, chiefly among children under 5. Nonetheless, even after surviving this, a large proportion of adults remain susceptible to infection and show periodic parasitemia, while their serum contains antiplasmodial antibodies, some with demonstrated protective action. Susceptibility to low-level infection provides the population with a protective **premunition** or prevention of subsequent infection during the course of a chronic asymptomatic current infection. In these hyperendemic areas of Africa, it is believed that nearly all residents harbor throughout their lives a continuous series of falciparum infections of low to moderate pathogenicity. The immune response that leads to protection is thought to be production of complement-independent antibody that inhibits entry of merozoites into the host erythrocytes. All immunoglobulin classes are elevated in the serum of malaria patients, but IgG levels appear to correlate best with the degree of malaria protection (or control of acute manifestations).

By various protective adaptations (eg, antigenic variability), the parasites survive and—by reason of a limited level of red cell destruction—elicit only a mild host response. Therefore, innate and acquired protective host responses (specific and nonspecific) and parasite counteradaptations to these host characteristics occur. In addition, vector biology and population fluctuation, as well as the parasite's response to varied ecologic conditions (such as a required ambient temperature for successful development of the sporogonic cycle in the mosquito), add to the vagaries of differential host/parasite survival. They combine to determine the host-parasite-environment balance in which each of the many patterns of human malaria exists.

Immunization has become a major focus of malaria research, accelerating with Trager and Jensen's breakthrough in 1976 of a continuous culture method for producing red cell asexual stages in vitro. Two approaches were originally followed: (1) induction of immunity using sporozoites inactivated by ultraviolet light, formalin, or mechanical disruption; and (2) the use of frozen or stored merozoites (which are spontaneously inactivated in 1 hour, so that special treatment is unnecessary). The first method induces a short-term thymus-dependent species- and strain-specific immunity active only against the sporozoite-induced infection. One approach involves use of sporozoites dissected from irradiated mosquitoes or recovered from the area of the bite of irradiated mosquitoes. Only mature infective sporozoites are immunogenic; adjuvants appear to be unnecessary. This method is limited by the difficulty in storing the vaccine, inability to culture and therefore obtain large amounts of immunizing antigen, the requirement of intravenous administration of the vaccine, and the continuing susceptibility of the immunized person to a merozoite infection (should any sporozoites succeed in developing in the liver).

Because of these problems, recent research has been directed toward developing a nonliving vaccine. If sporozoites are incubated in vitro with sera from vaccinated animals, a taillike immune precipitate is formed called the circumsporozoite reaction. This reaction is correlated with loss of parasite infectivity.

The target antigens of this reaction were first identified using monoclonal antibodies. These were found to be polypeptides that cover the entire surface membrane of the parasite, are species-specific, and contain repeating epitopes.

Subsequently, using recombinant DNA techniques, the genes encoding the circumsporozoite epitopes have been cloned and sequenced. An important finding was that the immunodominant epitope consisted of tandem repeated sequences of amino acids (asparagine-alanine-asparagine-proline in *P falciparum*). This discovery implied that a very simple vaccine antigen might be developed. In fact, synthetic peptides based on these sequences can elicit polyclonal antibodies that neutralize sporozoite infectivity.

The second approach to vaccine development—a killed or inactivated merozoite vaccine—induces an antibody that reacts with the red cell surface and selectively agglutinates infected red cells to produce a strain- and species-specific clinical cure. New infections can still develop, since there is no protection against sporozoites or the exoerythrocytic cycle. So long as the humoral titer is high, however, merozoites (but not gametocytes) will be destroyed, and symptoms will not develop. Rhesus monkeys, which normally are quickly killed by this form of malaria, were fully protected for 18 months when vaccinated with *Plasmodium knowlesi* merozoites. Freund's complete adjuvant is required—a major deterrent to development of a human vaccine. The synthetic adjuvant muramyl dipeptide is now used instead of Freund's adjuvant in rhesus monkey immunization studies. Helper T cells, other cell-mediated effector mechanisms, and humoral antibody are all involved. Extracellular merozoites are specifically inhibited by IgG and IgM in the absence of complement. Immunization in rhesus monkeys induces complete elimination of parasites after 1–3 weeks, whereas natural immunity following repeated infection and drug cure is associated with chronic relapsing parasitemia. Immunization probably is associated with far fewer soluble circulating antigens than occur in natural infection, which preferentially stimulates suppressor cells or lymphocyte mitogens, all of which favor parasite survival. Difficulties of immunization with a merozoite vaccine even with a nontoxic adjuvant include the risk of contamination of the merozoite vaccine with blood group substances acquired during its cultivation, and substantial potential problems of vaccine delivery, cost, and acceptance. Nonetheless, the possibility of a prophylactic or therapeutic merozoite vaccine is most promising.

TOXOPLASMOSIS

Major Immunologic Features

- Sabin-Feldman dye test positive.
- Specific antibody present.
- Nonspecific increase in serum immunoglobulins.
- Natural acquired immunity widespread; cell-mediated immunity probably the major means, aided by humoral factors.

Toxoplasma infection in humans is generally asymptomatic; it has been estimated that as much as 40% of the adult population in the world is infected, as well as all species of mammals that have been tested for the presence of this ubiquitous parasite. Clinical disease, which develops in only a small fraction of those infected, ranges from benign lymphadenopathy to an acute and often fatal infection of the central nervous system. The developing fetus and the aged or otherwise immunologically compromised host are most vulnerable to the pathologic expression of massive infection and resulting encystation in the eye or brain. Damage to the fetus is greatest during the first trimester, when the central nervous system is being organized, and nearly all such instances end in fetal death. Infection of the mother during the second trimester may produce hydrocephaly, blindness, or varying lesser degrees of neurologic damage. Most cases of fetal infection occur during the third trimester, resulting in chorioretinitis or other ophthalmic damage, reduced learning capacity or other expression of central nervous system deficit, or asymptomatic latent infection that may become clinically apparent years later. Women exposed *before* pregnancy—as indicated by a positive indirect immunofluorescent or Sabin-Feldman dye test—are thought to be unable to transmit the infection in utero.

Many potential sources of infection have been suggested, including tissue cysts in raw or partially cooked pork or mutton and oocysts passed in feces of infected cats (the true final hosts). Yet these sources seem insufficient to account for such large numbers of infections. The major reservoirs of infection are as yet unknown. *Toxoplasma* infection usually occurs through the gastrointestinal tract, and the protozoa can apparently penetrate and proliferate in virtually every cell in the body, though very rarely in mature red cells, forming cysts that remain viable for long periods. Following a cellular and humoral immune response, only encysted parasites can survive.

Toxoplasma infection results in production of IgA, IgG, and IgM antibodies, which can readily be demonstrated by hemagglutination, complement fixation, indirect fluorescent antibody techniques, and the Sabin-Feldman dye test (see Tables 35–1 and 35–2). The presence of antibody is not sufficient for protection, as shown by the ability of the parasite to persist in the presence of high antibody titers and by the fact that passive transfer of antibody is not protective.

The ability of macrophages to kill infective trophozoites is greatly increased if the parasites are first exposed to antibody and complement. This mechanism may be one way that parasite numbers are reduced in the infected host. The parasite can multiply only to a certain number within macrophages before the host cells are destroyed. When this occurs, extracellular trophozoites come into contact with antibody and may

be more efficiently killed by macrophages than before.

Cell-mediated immunity is also involved in protection against *Toxoplasma* because delayed hypersensitivity and its in vitro correlates such as production of migration inhibitory factor (MIF) develop early in toxoplasmosis, and protection results only from infection with *living* organisms. Interferon is also produced, and activated macrophages can be demonstrated that kill or inhibit multiplication of the parasite, which would effectively reduce the parasite burden. In such cellular immunity, macrophage activation is probably affected by the action of antigen upon specifically sensitized T lymphocytes, which in turn produce lymphokines that activate the macrophages.

An intact immune system is necessary for protection against *Toxoplasma;* thus, immunosuppression to control transplant rejection or malignancies or infection with the virus causing AIDS may result in active toxoplasmosis. This phenomenon may result either from the elimination of sensitized lymphocytes previously limiting an inapparent infection or from inability of the immunosuppressed host to mount an adequate protective response to new infection.

Vaccination of the population with strains of low virulence would probably be effective in establishing protection to *Toxoplasma,* but most persons develop adequate protection after natural infection. Although no such vaccine is available, the procedure probably would not be worthwhile, except for previously uninfected women of childbearing age to prevent intrauterine transmission.

AMEBIASIS

Major Immunologic Features

- Specific antibody detectable following tissue infection.
- Skin tests for immediate and delayed hypersensitivity indicate past or present disease.
- Delayed hypersensitivity depressed with liver abscess.

Immunity to amebiasis in humans remains unproved, though acquired immunity has been demonstrated in experimental animals, including dogs, hamsters, and guinea pigs—all unnatural hosts. Cases of repeated infection and repeated intestinal lesions in humans are common, even in the presence of high titers of circulating antibody. Antibodies of the IgG and IgM classes can be demonstrated by passive hemagglutination, precipitation, latex agglutination, and fluorescent antibody techniques. These antibodies can be used for diagnosis. However, the presence of specific antibody does not necessarily indicate active infection but rather prior exposure to the organism. Skin tests give immediate responses in many patients, indicating IgE production, and Arthus reactions can also be demonstrated. To date, there is little direct evidence that immunoglobulin is protective. See Trissl (1982) for a general review.

Cell-mediated immunity to *Entamoeba histolytica* antigens can be demonstrated by delayed hypersensitivity skin tests in many patients who do not have clinically evident disease, and recent evidence indicates that patients with amebic abscess of the liver have depressed cell-mediated immunity to amebic antigens while retaining their ability to respond to other skin test antigens such as streptokinase-streptodornase. Cell-mediated immunity returns after treatment for liver abscesses, and there is usually no recurrence of infection. It is not known whether this is due to protective immunity. Other normally nonpathogenic amebas such as *Naegleria* or *Acanthamoeba* can invade the central nervous system and cause rapid death due to meningoencephalitis *(Naegleria)* or local lesions in the throat or on the skin which may finally involve the central nervous system and produce death *(Acanthamoeba).* Probably little immune response to *Naegleria* occurs, owing to the brief survival of infected patients; however, *Acanthamoeba* may induce an immune response because of the duration of infection. No protective immune mechanisms to these parasites have been demonstrated. Diagnosis can be made from infected tissues by fluorescent antibody staining of the parasites. Amebiasis serodiagnosis can be performed by a number of techniques, including such procedures as counterelectrophoresis, gel diffusion precipitin, and enzyme-linked immunosorbent assay (ELISA).

LEISHMANIASIS

Leishmania is a genus of obligate intracellular parasites that infect macrophages of the skin and viscera to produce disease in both animals and humans. Sandflies, the principal vector, introduce the parasites into the host while taking blood meals.

A range of host responses interact with a number of parasite leishmanial species and strains to produce a panoply of pathologic and immunologic responses. Only in recent years have some of the factors responsible for the variety of disease manifestations and degrees of immunity to leishmaniasis been elucidated in experimental and clinical studies.

1. CUTANEOUS LEISHMANIASIS

Major Immunologic Features

- Delayed hypersensitivity present.
- Little or no specific serum antibody.

Old World cutaneous leishmaniasis, or tropical sore, is caused chiefly by various forms of *Leishmania—L tropica, L major,* and *L aethiopica.* These agents induce an immune response characterized by little antibody but strong cell-mediated immunity. In cutaneous leishmaniasis, it is chiefly the patient's immune response to the infection that determines the form taken by the clinical disease; however, the strain of parasite may also determine part of the host response. If the patient mounts an adequate but not ex-

cessive cell-mediated immune response to the parasite, healing of the ulcerative lesions and specific protection result. However, if cell-mediated immunity to the parasite is inadequate or suppressed, the result may be diffuse cutaneous disease, in which there is little chance of spontaneous cure. In the Old World, this condition is due chiefly to *L aethiopica* in East Africa. A similar form caused by *L mexicana pifanoi* occurs in Venezuela, again in specifically anergic patients. The cause of the specific anergy and whether it is host- or parasite-induced are unknown. On the other hand, an excessive cell-mediated immune response produces lupoid or recidiva leishmaniasis, caused by *L tropica*, in which nonulcerated lymphoid nodules form at the edge of the primary lesion; these lesions persist indefinitely, although parasites are not easily demonstrated. Thus, as in leprosy, a spectrum of host responses to cutaneous leishmaniasis exists, ranging from multiple disseminated parasite-filled ulcers (anergic response) to single, spontaneously cured immunizing sores, to recidiva hyperactive host responses with few or no parasites (allergic response). Parasite strain differences in virulence and other factors add to the complexity of the host-parasite interaction, resulting in prolonged disease or cure with immunity.

Delayed hypersensitivity ordinarily occurs early during the course of cutaneous leishmaniasis; nevertheless, new lesions can develop for several months. Secondary lesions quickly assume the histologic picture of the early lesions (the isophasic reaction) and usually heal at the same time as the primary lesion or shortly thereafter. Protection appears to be permanent after a primary infection has terminated naturally, although immunosuppressive treatment of patients residing in endemic areas has resulted in reinfection in previously protected individuals. It is not known whether this is due to new infection or to recrudescence of the old disease. If excision is used to terminate the primary infection before spontaneous healing has taken place, protection against reinfection may not occur. Animal experiments indicate that sensitized lymphocytes are widely distributed when the lesion heals. Thereafter, new disease presumably cannot occur, because immune lymphocytes are generally distributed in lymph nodes and spleen. Reinfection is usually manifested by a prolonged delayed hypersensitivity response at the site of the sandfly bite, but ulceration does not follow. Vaccination with virulent strains of the parasite is a common practice for cosmetic protection and to assure uninterrupted work in highly endemic areas, as in parts of southern USSR and Israel. "Vaccination" against *L major* is a full, lesion-producing infection in a selected skin area. Avirulent, modified, or dead parasites will not induce a protective response. In fact, only the most virulent strains will protect against the same and other strains; less virulent forms protect only against reexposure to the same strain.

Cell-mediated and humoral immune responses may act together to produce protection after initial infection.

2. VISCERAL LEISHMANIASIS

Major Immunologic Features

- Delayed hypersensitivity only after spontaneous recovery or chemotherapy.
- Increased nonspecific immunoglobulin levels.

The immune response to visceral leishmaniasis (kala-azar)—caused by various subspecies of *Leishmania donovani* (considered separate species by some authors)—is remarkably different from that of cutaneous leishmaniasis, although the parasites are essentially indistinguishable. Massive polyclonal hypergammaglobulinemia with little or no evidence of cell-mediated immunity is the rule in visceral leishmaniasis. There is no quantitative relationship between the elevated serum immunoglobulin and antiparasite antibodies, which are, moreover, not species-specific. The elevated immunoglobulin diminishes rapidly when treatment begins. Delayed cutaneous hypersensitivity to parasite antigens becomes demonstrable only after spontaneous recovery or treatment, which suggests that cell-mediated mechanisms play a role in the resolution of the infectious process. Under certain circumstances, post-kala-azar dermal "leishmanoid" occurs. Nodules containing many parasites form papules as a result of incomplete or defective cell-mediated immunity, or a persistent allergic reaction to parasite antigens. Most cases have been reported from India, developing 6 months to 2 years after cure of kala-azar. Insufficient data are available at this time to establish exact correlation between delayed hypersensitivity and protection. Serodiagnosis is readily available by indirect hemagglutination, immunofluorescence, complement fixation, direct agglutination, and ELISA.

3. AMERICAN LEISHMANIASIS

Major Immunologic Features

- Positive delayed hypersensitivity.
- Increased immunoglobulin levels.
- Anergic type of host response.

Cutaneous leishmaniasis of the New World is caused by a number of leishmanial pathogens now divided into 2 species complexes: *Leishmania mexicana* (subdivided into 4 or more subspecies) and *Leishmania braziliensis* (subdivided into 4 or more subspecies). The parasite subspecies are distinguished on the basis of growth characteristics in the vector and in culture, isoenzyme electrophoresis patterns, kinetoplast DNA analysis, lectin-binding specificities, excreted factor serotyping, and monoclonal antibody probes. Also taken into consideration in typing are geographic factors, hosts, and the character of the disease produced in humans.

The most significant clinical distinction in the *L mexicana* complex is the high frequency of ear cartilage lesions (chiclero ulcer) and rare diffuse cutaneous

leishmaniasis; in the *L braziliensis* complex, the development of metastatic lesions, usually within 5 years of healing of the initial ulcer, which itself may be large, persistent, and disfiguring. Nasal cartilage and other nasopharyngeal tissues are attacked and destroyed by this subsequent massive ulceration (espundia), which may erode away much of the face and cause death by septic bronchopneumonia, asphyxiation, or starvation. This manifestation of American leishmaniasis is frequently nonresponsive to treatment. Parasites are abundant in the early stages of espundia but subsequently are rare, while persistent infiltration of giant cells, plasma cells, and lymphocytes is characteristic. Delayed and perhaps immediate hypersensitivity and circulating antibody levels are higher in espundia than in cases of the primary lesion alone. The mucocutaneous form is thought to be an allergic or abnormal immunologic manifestation of infection with the type subspecies *L b braziliensis*. Both host and parasite genetic factors—as well as vector characteristics—appear to be involved in this additional example of the interaction between immunogenicity of the parasite and immunologic response of the host.

A skin test (Montenegro test) is rapidly positive with cutaneous leishmaniasis, particularly the New World forms. Dermal response to kala-azar is slower, becoming positive only after cure of the visceral infection. Serodiagnosis of cutaneous leishmaniasis is still unsatisfactory because of low serum antibody levels and, in Latin America, because of cross-reactions with Chagas' disease antibodies.

TRYPANOSOMIASIS

1. AFRICAN TRYPANOSOMIASIS

Major Immunologic Features

- Increase in nonspecific IgM.
- Succession of parasite populations in bloodstream, each with a different antigenic coating.

Trypanosoma brucei gambiense, also called *T gambiense*, is the agent of chronic Gambian or West African sleeping sickness. *Trypanosoma brucei rhodesiense*, also called *T rhodesiense*, is the agent of acute Rhodesian or East African sleeping sickness. Both cause human disease, and the Rhodesian form is most responsible for denying vast areas of Africa to human occupation, chiefly in the flybelt regions where the tsetse fly vectors are found. Tsetse-borne trypanosomes (*Trypanosoma b brucei* as well as several other species) infect domestic animals with similar or even greater virulence. The impact of this dual threat—one to humans and the other to domestic animals, especially cattle—has had an enormous effect on human history in Africa and the occupation of vast regions across the tropical belt from West African forests to the savannahs of East and South Africa. The great herds of wild herbivores, once abundant everywhere, have survived in this region because of their natural tolerance to heavy infections. The trypanosomes multiply extracellularly in successive waves in the human and animal bloodstream but produce very little disease in spite of their numbers. Only when the parasites enter the central nervous system does the ravaging disease sleeping sickness develop. It is this pathologic phase of an otherwise harmless chronic or recurrent infection to which humans and domestic animals succumb and which most native antelope and other herbivores resist. Presumably, this is a result of association over millions of years accompanied by a continuing strong natural selection process.

Greatly increased levels of immunoglobulins, especially of the IgM class, are regularly present in infected humans and animals. The increased immunoglobulin levels, which do not correlate positively with protection, may result from B cell mitogens produced by the trypanosomes themselves or by the increased IgG production of helper T cells which act nonspecifically to increase immunoglobulin levels. A large proportion of the immunoglobulin in infected hosts is nonspecific in nature.

Despite the fact that trypanosomes are continually exposed to the host immune system in the bloodstream, they evade the host's defenses. The first hint of how this is accomplished was noted in 1910, when the periodicity of fever in patients with trypanosomiasis was correlated with a sharp rise and fall in the number of trypanosomes found in the blood. More recently, it was discovered that when individual organisms are cloned in culture, each clone displays a unique antigenic surface protein. When organisms first enter the host (Fig 35–2), the host immune system generates antibodies against the predominant surface antigen (variable surface glycoprotein; VSG). Antibodies can kill over 90% of the original infecting trypanosome population. The reason not all of the trypanosomes are killed is that some have switched on a different VSG antigen not recognized by the initial immune response. This switch occurs spontaneously and can be detected in immune-deficient mice. It is therefore not dependent upon the host immune response. The switch occurs very rapidly, so that by 5 days into an infection parasites with more than one antigen type can be detected. By 6 days, as few as 15% of the trypanosomes may still have the initial surface VSG. This switching from one VSG to another explains the waves of parasitemia and periodicity of the fever characteristic of trypanosomiasis. The potential VSG repertoire is not known, although parasites derived from a single parent trypanosome have been found with more than 100 distinct VSGs.

What is the mechanism by which the trypanosome can so quickly switch its surface coat? Recombinant DNA techniques have been used to unravel part of the mystery. Partial or complete nucleotide sequences have been determined for several cDNAs (DNA complementary to the messenger RNA) for VSGs. From these DNA copies of the messenger RNA, the amino

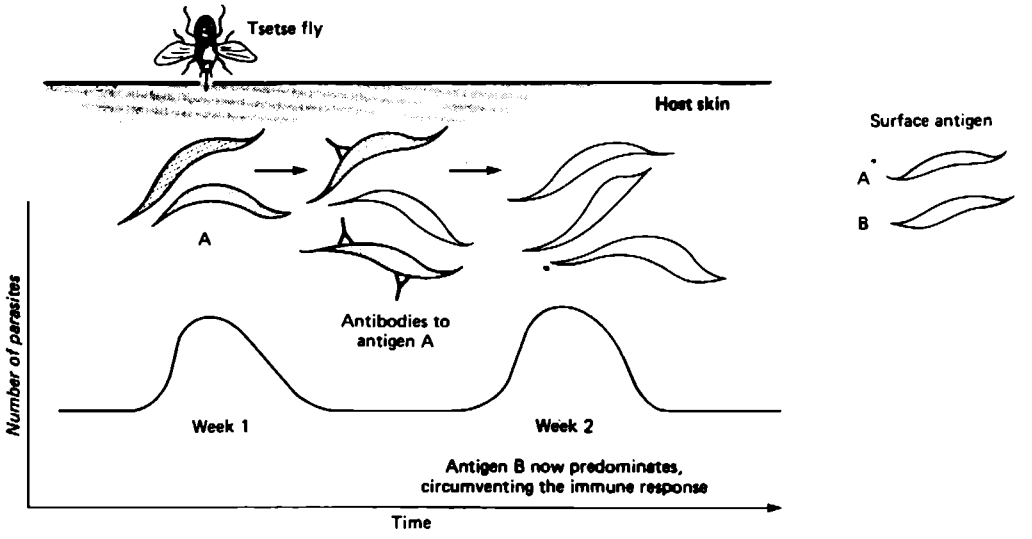

Figure 35–2. Antigenic variation and parasitemia in trypanosomiasis.

acid sequence of the VSG itself can be deduced from the genetic code. The first 20–30 amino acids constitute what is known as a "signal peptide." This peptide directs the newly synthesized VSG across the trypanosome's cell membrane. The middle 360 amino acids are quite different in each VSG and are therefore responsible for the diversity of surface antigens. The last 120 amino acids, at the carboxy-terminal end of the protein, are quite similar, and 20 of them are clipped off and replaced by a complex sugar molecule that anchors the VSG to the cell membrane.

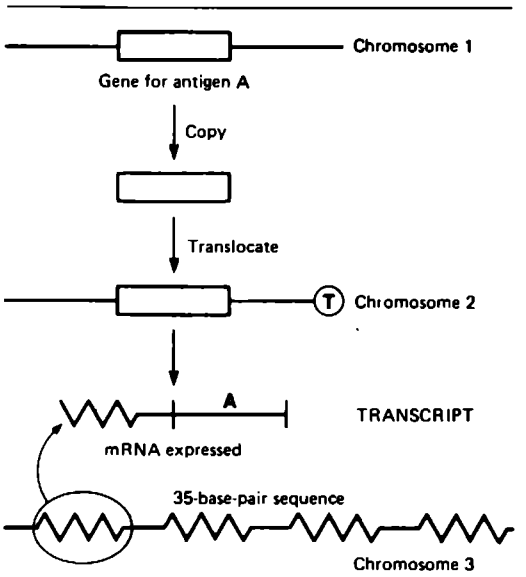

Figure 35–3. Molecular mechanism of antigenic diversity in trypanosomiasis.

The actual mechanism by which the trypanosome switches on one VSG is quite elegant (Fig 35–3). One copy of the VSG gene is located on a specific trypanosome chromosome. If that VSG is to be expressed, a copy is made of the gene, and it is translocated to another chromosome close to the telomere. In this new location—and only in the new location—it is transcribed into messenger RNA to which a 35-nucleotide sequence is added. This small 35-nucleotide sequence has been transcribed from yet another site where many of these small sequences are found closely linked to each other. Most trypanosome proteins have this small sequence at the beginning of their message. Therefore, it is assumed that it is necessary for expression of the messenger RNA. The copy of the VSG gene that is not expressed is called the "basic copy gene." However, some of the VSGs come from genes that are already near the telomere and therefore do not translocate before expression. Although the mechanism of "gene jumping" and subsequent expression has been elucidated, the exact mechanism by which one VSG is switched to another is still unclear. Understanding of this switching mechanism might provide a means of interrupting the ability of trypanosomes to change their antigenic disguises.

Serodiagnosis of trypanosomiasis is possible using indirect immunofluorescence. ELISA, indirect hemagglutination, direct agglutination, gel precipitation for IgM titration, and gel precipitation using trypanosomal antigen. However, none of these methods are yet suitable for field studies or surveys in Africa.

Specific antibodies to trypanosomes can either lyse the parasites or clump them. Clumping allows for more efficient removal of the parasites by the reticuloendothelial system. It is controversial whether humans or domestic animals living in endemic areas develop resistance to infection, although epidemiologic

observations suggest that resistance does arise. The fact that there are healthy human carriers of *T rhodesiense*—which usually produces a fatal infection—implies that some protective mechanism must exist. However, the precise immunologic nature of this protection is at present obscure.

Suppression of immune responses to other unrelated antigens may be observed during trypanosomal infections. It is not known whether the suppression results from exhaustion of B cells, the presence of suppressor T cells, a lack of helper T cells, or the availability of fewer T cells to interact with new antigens.

The multiplicity of antigenic variants observed during field studies in bovines makes vaccination an unlikely solution to trypanosomiasis unless common antigens can be found.

2. AMERICAN TRYPANOSOMIASIS

Major Immunologic Features

- Specific antibody not necessarily indicative of active infection.
- Delayed hypersensitivity present.
- No antigenic variation.

An estimated 24 million people in Central and South America are infected with *Trypanosoma cruzi*, and 50 million more are at risk. The resulting chronic debilitating affliction, Chagas' disease, has no cure and is a major factor in premature death from heart disease in Latin America—especially in rural areas where housing and nutrition are inadequate. The disease is transmitted to humans when fecal contamination from infected bloodsucking triatomine bugs occurs in fresh bites, mucous membranes, or abraded skin, frequently from nighttime scratching that rubs the vector's liquid feces into the bite or causes it to be taken into the mouth or rubbed into the eyes. Accidental laboratory infections from sprayed culture material or chance injections have also occurred. The parasites actively penetrate host cells and multiply intracellularly. The acute form of the disease is characterized by myocardiopathy, lymphadenopathy, hepatosplenomegaly, parasitemia, fever, and malaise. Colonies of intracellular amastigotes develop in striated muscles, smooth muscles, and the reticuloendothelial system. The disease is often fatal in infants or children, but in adults a chronic form usually follows the initial infection, sometimes after a considerable interval, producing a disease characterized by cardiac enlargement with megacolon, megaesophagus, and degeneration of the peripheral and central nervous systems; this is found in parts of Brazil. In chronic disease, nests of intracellular parasites can be found, but parasitemia accompanied by fever is infrequent.

Serologic tests include complement fixation (still the test of choice), latex agglutination (for rapid screening), indirect hemagglutination (for epidemiologic studies using samples collected on filter paper, which is also suitable for the ELISA test), and the direct agglutination test (sensitive for congenital infection). All have some cross-reactions, especially with *Leishmania*. A thin-layer immunoassay is comparable to ELISA and does not require a labeled reagent. Monoclonal antibodies have been characterized. Antibody may persist after infection and therefore does not indicate active disease. High titers of antibody do not appear to limit the infection in humans; however, complement-dependent lysis of the parasites can be demonstrated in vitro with sera from experimentally infected hosts. This lysis may be an important mechanism for parasite control in vivo. However, lytic antibody does not afford complete protection against reinfection, since passive transfer of hyperimmune serum is not always effective. The lytic action of antibody may need cooperation with cells such as macrophages to eliminate or lower the parasite burden. Animals can be protected against virulent strains of the organism by infection with avirulent or partially virulent strains or by passive transfer of sensitized lymphocytes. Acquired resistance to virulent strains is probably the result of previous inapparent infection with strains of low virulence. Therefore, progress toward chemically attenuated vaccines is being made.

Activated macrophages can be demonstrated in *T cruzi* animal infections. There is some evidence from animal experiments that activated macrophages play the major role in protection against this infection. Delayed hypersensitivity, lymphocyte activation, and MIF production can be documented during infection, but the role of cell-mediated immunity has not been fully elucidated. Additionally, transfer factor made from the leukocytes of immunized monkeys can apparently induce certain new cell-mediated functions. Cardiac damage in infected rabbits may be the result of an immune response to cross-reacting antigens of *T cruzi* and rabbit cardiac muscle, since *T cruzi*-sensitized lymphocytes are cytotoxic for unparasitized cardiac muscle cells in vitro as well as for parasitized cells. Antibody may also participate in immune destruction of normal cells. Attempts to vaccinate against disease might do more harm than good if such sensitized lymphocytes against shared antigens should develop.

THE IMMUNE RESPONSE TO HELMINTHS

From the foregoing, it is obvious that the immune response to unicellular parasites is complex. Multicellular parasites, by reason of their size, more complex tissue and organ structure, and varied and active metabolism induce even more complex host responses. Further complicating the picture is the fact that several forms of the parasite may be present in the host, each eliciting a unique immune response.

The primary antigens of helminths may often be

metabolic by-products, enzymes, or other secretory products rather than structural components. For example, the eggs of *Schistosoma mansoni* have been shown to secrete unique antigens that induce granuloma formation; the various stages of developing nematodes have stage-specific antigens, often molting fluids, to which the host responds in various ways; and the granules in the stichocytes, special cells located in the "neck" of *Trichuris trichiura*, elicit specific antibody.

Nematodes, cestodes, and trematodes probably all share common antigens. The 2 most frequent responses to helminths—eosinophilia and reaginic antibody (IgE)—are both T cell-dependent (see Chapter 15). Moreover, certain helminths have been shown to potentiate the immune response to other antigens, perhaps by common metabolic by-products acting as nonspecific adjuvants. In addition, helminthic infection often induces strong and sometimes self-destructive immunopathologic reactions, such as the excessive granulomatous response to schistosome eggs caught in host tissues.

TREMATODES

Trematodes are important pathogens of humans and domestic animals. Fascioliasis debilitates and kills domestic animals in large numbers and renders the livers unfit for human consumption. Schistosomiasis is a major disease of humans. The lung flukes of the genus *Paragonimus* cause central nervous system complications in humans if they encyst in the brain. In the lung, considerable mechanical damage results. *Clonorchis sinensis*, the fish-borne Chinese liver fluke, causes much morbidity both in the Orient and among recent emigrants from endemic areas, producing infection that may last the lifetime of the host.

1. SCHISTOSOMIASIS

Major Immunologic Features

- Response to invading worms is both humoral (IgE, IgM, IgG) and cellular (eosinophils, macrophages).
- A serum sickness-like acute disease may develop (Katayama fever).
- Chronic disease due to granulomatous reaction to eggs with subsequent fibrosis.
- Developing larvae and adult worms evade immune response by camouflaging their surface with host antigens.

Schistosomiasis in humans is caused by *Schistosoma mansoni*, *S japonicum*, *S haematobium*, and *S mekongi*. The advent of new high dams in many areas of the world, especially Africa, has increased the prevalence of schistosomiasis, because the additional irrigation made possible by the dams has vastly enlarged the habitat of the freshwater snails that serve as intermediate hosts to the worms. The life cycle of this parasite depends upon skin penetration of the definitive host by infective larvae produced in large numbers in the snail. Because attempts to reduce snail populations have largely failed, infection has become rampant in these areas. *S mansoni*, now widespread in Africa and the Middle East, has also spread extensively in South America. *S haematobium* is found in all watered areas of Africa and the Arabian peninsula. *S japonicum* is found in the Yangtze River watershed in China, where it has been subjected to a vast control effort but is still common in Szechwan province and may be returning to the main river valley. It is also common in the central Philippines. A purely animal-infecting (zoophilic) form is found in Taiwan. *S mekongi*, a newly described species similar to *S japonicum*, causes human disease in Thailand, Laos, and Cambodia, with a scattering of cases in Malaysia. There is also a focus of *S japonicum* in Sulawesi (Celebes) that may prove to be a distinct species.

In brief, the life cycle of the schistosomes that infect humans is as follows: Infected humans and animals excrete eggs that hatch in water, releasing miracidia; these actively penetrate snails in which several generations of multiplying larvae (sporocysts) develop. These in turn produce great numbers of fork-tailed cercariae, the stage infective for humans, which leave the host snail at the rate of 300–3000 per day. The cercariae penetrate the skin of the definitive host, leaving the tail outside, and enter the bloodstream as minute motile immature schistosomula, which migrate in 3–8 days to the lungs and eventually to the liver. Further development and adult worm pairing take place about 5 weeks after skin penetration. The paired mature schistosomes then migrate against the venous flow into the mesenteric or vesical venules, where eggs are deposited. The embryo (miracidium) within the egg secretes proteases to facilitate passage through the blood vessel and adjacent tissue into the lumen of the intestine (or bladder in the case of *S haematobium*). Egg movement is probably aided by peristalsis of the intestine or contractions of the bladder.

Unfortunately, not all the eggs reach the lumen of the intestine or bladder. Some become trapped in the submucosa, and others do not leave the bloodstream but instead are carried with the portal venous flow to the liver, or by collateral circulation to other organs of the body. Because of their size, eggs reaching the liver become trapped in the portal venules and do not enter into the sinusoids. When eggs are trapped in the liver, the wall of the intestine, or the bladder, they elicit a granulomatous inflammation that is the hallmark of the chronic stage of schistosomiasis. In experimental animals, an early infiltrate of neutrophils and lymphocytes may be seen around eggs, but distinctive granulomas containing a core of macrophages and eosinophils, surrounded by a cuff of lymphocytes, appear shortly. In experimentally infected mice, the early stages of inflammation may be seen by 6 weeks, but granulomas reach their maximal cellularity within

9–12 weeks. Later, an increasing number of fibroblasts can be seen in association with the granulomas, and the cellular lesion becomes slowly replaced by collagen. In the liver, older lesions become periportal scars. Since numerous eggs are deposited, a circumferential periportal fibrosis called Symmer's clay pipestem fibrosis develops. This fibrosis blocks normal blood flow from the portal venous system to the sinusoids, resulting in portal hypertension and its complications.

The factors that initiate granuloma formation are soluble proteins secreted through pores in the eggshell by the embryonic miracidium. These soluble egg products, some of which have been purified, are used in one serodiagnostic test for schistosomiasis. As might be expected from the complex group of cells comprising the granuloma, the mechanisms of granuloma formation, modulation, and subsequent fibrosis are complex. Interleukins and lymphokines have been identified, as well as factors from the egg itself that may be both chemotactic and mitogenic for fibroblasts.

While the immune response to schistosome eggs is the central immunopathologic mechanism in chronic schistosomiasis, it is not the only immune response of importance in schistosome infection. In some previously infected individuals, invading cercariae may elicit a dermatitis with features of both immediate and delayed hypersensitivity. This is similar to the "swimmers' itch" produced by nonhuman schistosomes. Some schistosome species may produce an acute form of schistosomiasis (Katayama fever) characterized by fever, eosinophilia, lymphadenopathy, diarrhea, splenomegaly, and urticaria. This appears to be an anaphylactic (IgE) or serum sickness (IgG) reaction. In fact, cases of glomerulonephritis secondary to schistosome antigen-antibody complexes have been reported.

An important unresolved question is whether immunity to schistosomiasis develops in humans after infection. Studies in Kenya and Gambia have shown that schistosome-infected individuals in an endemic area who had been treated with antischistosome drugs showed an age-dependent resistance to reinfection. Children were much more easily reinfected than were adults, suggesting that true human immunity can be acquired with age. However, these conclusions remain controversial because the studies were retrospective and because it is difficult in field studies to control for important factors such as the amount of water contact. Nevertheless, identification of "resistant" groups of children may help to identify important parasite antigens. Augmentation of the response to these antigens would be one rational approach to vaccine development.

The question of immunity to reinfection has been studied intensively in animal models. Two models in particular have been used. In the first of these, called the **concomitant immunity model,** mice are infected with 20–30 normal *S mansoni* cercariae 6 weeks prior to challenge. In the second, called the **attenuated vaccine model,** mice are immunized by 400–500 cercariae attenuated by 20–50 kilorads of gamma radiation 2 weeks before challenge. For many years it was thought that the resistance to reinfection that developed in a concomitant immunity model was due to circulating antigens from the established adult mating pairs causing a heightened immune response to newly invading larvae. However, it has recently been shown that the principal reason new larvae do not develop is alterations in the lung caused by the granulomatous response to eggs laid by resident adult females. The granulomatous response to eggs that have reached the lung circulation and the subsequent fibrosis alter blood flow through the lung. Since schistosomula must pass through the lung circulation en route to their eventual residence in the portal venous system, any alteration in normal blood flow through the lung would increase parasite attrition. Because of this finding, interest has turned to the attenuated vaccine model for insights into the host immune response to larvae.

By comparing normal with various immune-deficient mouse strains, the cellular and antibody requirements of vaccine immunity have been investigated. Vaccinated mice with T lymphocyte deficiencies, as well as mice immunosuppressed from birth, have a sharply diminished resistance to a challenge infection. On the other hand, mice deficient in complement, mast cells, NK lymphocytes, and IgE show no difference in resistance compared to normal controls. Macrophages appear to be key effector cells in the resistance of vaccinated mice to challenge infection.

The exact site of killing of schistosomula in vaccinated mice remains controversial. Some evidence exists for immune killing in the skin, while other studies indicate that most killing occurs in the lung.

Whereas acquired human immunity to schistosomiasis remains unproved, there are many examples of natural resistance to infection among mammals. Chimpanzees show little or no evidence of acquired immunity, and the disease produced is strikingly like that in humans. Baboons develop a slowly acquired immunity, while the rhesus monkey develops a spontaneous "self-cure" several weeks after infection. The grivet monkey develops both an acute illness and a strong resistance to reinfection.

A similar broad range of responses can be demonstrated in rodents. The rat is an excellent model for high natural resistance, spontaneous decrease of worm burden in about 1 month, and increased resistance from initial stimulation of T cell-dependent and then B cell-dependent mechanisms. There is an anamnestic response to normal or irradiated cercariae or to worm homogenates, but intact viable parasites are very immunogenic, and immunity is especially strong against younger stages. Strong adoptively transferred resistance using cells *or* serum can also be demonstrated.

The mouse is the principal host for maintenance of the worms, as it is considerably more susceptible, produces a less active immunity, and demonstrates far more disease—rather like the human response. The ef-

fects of IgG and polymorphonuclear leukocytes against young schistosomula are strongly evident in the rat, whereas IgE and eosinophil effects are especially prevalent in mice and humans.

Immunodiagnosis of schistosome infection in the absence of egg excretion by the host can be accomplished in various ways, both humoral and cellular. Stage-specific humoral responses can be used to produce circumoval precipitation, schistosomule growth inhibition or death, and complement fixation, hemagglutination, and various precipitation reactions. None of these reactions can be positively correlated with protection. Immediate and delayed cutaneous hypersensitivity develop in most individuals during the course of the disease, although the specificity of these reactions is often suspect, owing to antigens that cross-react with those of other worms. However, the purification of novel antigenic fractions and sensitive ELISA or radioimmunoassays may improve the specificity of these responses.

Major efforts are now under way to develop a nonliving vaccine for schistosomiasis. This goal is being approached in a variety of ways. Some laboratories are identifying antigens recognized by serum from patients infected with schistosomes and then attempting to clone the genes coding for these antigens. This would allow unlimited production of polypeptide antigen for augmentation of the immune response. Another approach involves identifying groups of individuals in endemic areas who appear to show heightened resistance to the disease. Antigens unique to these groups are then searched for and characterized. A third approach involves purification of proteins critical for the metabolism and development of the parasite within the host and testing purified protein for immunogenicity. All of these studies are still very early but represent one of our best hopes for control of the disease.

2. CERCARIAL DERMATITIS (Swimmers' Itch)

The invasion of a previously sensitized host by the cercariae of schistosomes, particularly those of avian origin, can cause severe 2-stage reactions in the skin. The first stage begins within minutes of contact and consists of a wheal-and-flare reaction. The second stage becomes evident 16–24 hours after contact, with development of papules which are essentially delayed hypersensitivity reactions. These reactions have been shown to be very specific in that persons infected with *S mansoni* did not react to cercariae of an avian schistosome known to cause violent reactions in persons with swimmers' itch.

CESTODES

There are 2 types of immune response to cestodes. One is directed against the intestinal lumen-dwelling adult tapeworms such as *Diphyllobothrium latum* and *Taenia saginata*, which have restricted, nonhumoral immunogenic contact. The response is chiefly cell-mediated, is induced primarily by the scolex, affects growth and strobilation of challenge worms, and varies considerably with the host species. The other is directed against migratory tissue-encysting larval tapeworms such as *Hymenolepis nana* (in its intravillous larval phase), *Echinococcus granulosus* (hydatid cyst), and *Taenia solium* (cysticercosis), which have intimate and continuous tissue contact and induce a strong parenteral host response detectable as serum antibody and strongly protective against reinfection. Serodiagnostic tests are available only for the larval tissue cestode parasites, and humoral responses that protect the challenged host have only recently been described for this form of cestode parasitism. Development of ELISA for serodiagnosis of cysticercosis—with some cross-reactivity—has been accomplished.

Infection with cestodes is usually life-threatening to humans only when they act as unnatural intermediate hosts. *T solium* larvae normally develop as cysticerci in swine, but eggs from adult worms in the human intestine can pass via fecal contamination to other humans. They may then hatch, penetrate the gut wall as 6-hooked larvae, and pass to any organ of the body, inducing the extraintestinal disease cysticercosis. When encysted parasites die in the brain, severe tissue reactions with resultant central nervous system disorders and pressure damage can occur. Death may result, depending upon degree of toxicity and tissues affected.

ECHINOCOCCOSIS

Major Immunologic Features

- IgE elevated.
- Anaphylaxis due to ruptured cyst fluids.
- Casoni skin test of questionable use.
- Diagnostic antibody present.

The most serious human cestode infection is that caused by *Echinococcus*. These tiny tapeworms do not produce pathologic lesions in the definitive host, the dog, but severe complications occur when their eggs are ingested by humans and other animals. The larval form of the tapeworm hatches from the egg in the intestine of the intermediate host, eg, humans, and then claws its way through the intestinal mucosa and is transported through the lymphatic and blood vessels to sites in which it grows to enormous proportions though it is enclosed by a heavy cyst wall laid down by both the host and by the parasite. In humans, *Echinococcus* normally forms fluid-filled cysts in the liver, but these can also occur in the lungs, brain, kidneys, and other parts of the body. Hydatid cysts are highly immunogenic and result in production of high titers of reaginic antibody (IgE) and other immunoglobulins. If a cyst is ruptured, anaphylactic response to the cyst fluid can cause death. Little or no protection seems to be elicited by this highly immunogenic cestode be-

cause the hydatid cysts remain alive for years and in animals can be shown to increase in number as the host ages. Humans are usually a dead-end host, for the cysts must be eaten by a canid to become sexually mature. There is some evidence that complement-mediated lysis of protoscoleces (the numerous future scoleces in hydatid fluid or "hydatid sand") might be protective in the infected human or other intermediate host.

The Casoni skin test indicates past or present echinococcosis. It consists of intradermal injection of hydatid cyst fluid, resulting in both immediate and delayed hypersensitivity. The specificity of this test is in doubt because of cross-reactions with other helminths. Heating the cyst fluid slightly increases the specificity of the test. Serodiagnosis can be made by hemagglutination, complement fixation, and flocculation tests, ELISA, and radioimmunoassay, using serum of the patient and specially fractionated antigenic components made from cyst fluid. These tests are not species-specific.

NEMATODES

Nematodes are the commonest, most varied, and most widely distributed helminths infecting humans. As with other parasites, immunogenicity is a reflection of degree and duration of parasite contact with the host's tissues. Even with the intestinal lumen dwellers such as *Ascaris* there is a migratory larval phase in which such contact is made—in most cases, in the pulmonary capillaries and alveolar spaces. The hookworms of humans *(Ancylostoma duodenale* and *Necator americanus)* also undergo a migration except that the infective larvae enter via the skin or buccal mucosa rather than as hatchlings in the small bowel. *Strongyloides stercoralis,* the small intestinal roundworm of humans, undergoes a similar hookwormlike migration (as well as a stage of internal autoreinfection or reinvasion via the mucosa of the large intestine). *Trichuris trichiura,* the human whipworm, and *Enterobius vermicularis,* the pinworm, do not undergo parenteral migration; thus, their immunogenicity is limited to direct interchange between worm and host mucosa. In the case of the adult whipworm, its hairlike anterior end contains a row of stichocyte cells whose products are thought to be strongly immunogenic. The anterior end ("whip") becomes deeply embedded in the mucosa of the large intestine, as is also true of the entire bodies of the much smaller *Strongyloides* worms. Only the pinworm lacks a strong or prolonged contact with host tissues, and ready reinfection is the rule rather than the exception among children exposed to the eggs of this ubiquitous urban-adapted parasite. Work with rodent pinworms suggests that increasing resistance with age is thymus-dependent.

Living worms characteristically are required in order to induce functional (as opposed to measurable) immunity. The immature stages are particularly immunogenic, probably because of their high production of antigens from secretory glands and of enzymes or other products from these metabolically active stages. Commercially prepared vaccines are available only for nematodes, and all are living larval worms, irradiated to arrest their development but not their immunogenic activities. These are the cattle and sheep lungworms *Dictyocaulus viviparus* and *Dictyocaulus filaria* and the dog hookworm *Ancylostoma caninum.* A protease found in *A caninum* adults and larvae has been purified and shown to be immunogenic. Large amounts of polypeptide have been produced from a cDNA clone. Since it probably functions in both adult feeding and larval invasion, this enzyme is a promising serodiagnostic and vaccine agent.

Serologic studies of human nematodes have focused on *Trichinella spiralis,* the agent of trichinosis. These parasites have a tissue phase in 2 life cycle stages, one with the developing adult within the intestinal mucosa and then with the encysted larva within a muscle cell, which is converted by the parasite's presence into a "nurse cell." Not only is immunogenicity particularly strong with the *Trichinella* worm, but serodiagnosis is the best available diagnostic tool, since eggs or larvae are not routinely passed in the stool. Another parasite receiving special serodiagnostic study is the dog ascarid *Toxocara canis,* an agent of visceral larva migrans. This organism is acquired, usually by children, as embryonated eggs in contaminated soil. They hatch in the gut, penetrate the submucosa, enter the bloodstream, and eventually migrate in human viscera until they die or are encapsulated, sometimes 2 years later. This parasite is not normally infectious to humans and cannot complete its life cycle there, as can the human ascarid, *Ascaris lumbricoides.* Serodiagnosis of visceral larva migrans is therefore the only reliable diagnostic tool available.

Ascaris has been the subject of considerable research into hypersensitivity induced by migratory larvae in the lung or even airborne antigens from adult worms, as may occur in the biology laboratory. Another important group of human parasites are the filariae (chiefly *Wuchereria bancrofti, Brugia malayi, Loa loa, Onchocerca volvulus,* and the related guinea worm, *Dracunculus medinensis*). Diagnosis of these infections is often difficult, and various hypersensitivity reactions, especially after treatment, are common. Serodiagnosis of these infections is unsatisfactory—in part because of the presence of common antigens and the absence of highly specific immunologic tests. Use of monoclonal antibodies offers a new approach to diagnostic specificity.

1. TRICHINOSIS

Major Immunologic Features

- Positive skin tests for immediate and delayed hypersensitivity.
- Diagnostic antibody present.

Trichinosis is acquired by ingestion of the infective larvae of *Trichinella spiralis* in uncooked or partially

cooked meat. Pork is the primary source of infection in humans. The larvae are released from their cysts in the meat during digestion and rapidly develop into adults in the mucosa of the host's small intestine. After copulation in the lumen, the males die and the females return to the intestinal mucosa where for about 5–6 weeks they produce 1000–1500 larvae per female, which migrate through the lymphatic system to the bloodstream. These larvae travel in the blood to all parts of the body and develop in voluntary muscles, especially in the diaphragm, tongue, masticatory and intercostal muscles, larynx, and the eye. Within the sarcolemma of striated muscle fibers, the larvae coil up into cysts the outer walls of which are rapidly laid down by host histiocytes. Larvae may remain viable and infective for as long as 24 years even though the cysts calcify. The encysted larvae apparently do not yield protection. The migrating larvae and adult forms of the parasite excrete antigens that appear to be responsible for protection from subsequent challenge infections. An important expression of host resistance is active expulsion of developing or adult worms from the gut of a parasitized host—the so-called self-cure phenomenon. This occurs when a new infection initiates a host response, resulting in elimination of the old one—the opposite of concomitant immunity. Much work on this topic has been done with nematodes of veterinary importance, such as the highly pathologic stomach worm of sheep, *Haemonchus contortus*. It has also been reported with *Ascaris* in pigs and humans, *Toxocara* in dogs, and *Brugia pahangi* in cats. The reaction appears to be immediate hypersensitivity induced by antigens released from developing larvae of a new infection, which initiates an anaphylactic reaction resulting in cessation of egg production and even worm expulsion. However, experimental confirmation is lacking, and the inconsistency of this reaction requires explanation. Of special interest is the fact that some of the incoming larvae appear to escape and continue to develop to adult worms in spite of elimination of the preceding generation.

The expulsion of *T spiralis* in humans appears to follow the mechanism proposed by Ogilvie and coworkers for the *Nippostrongylus brasiliensis* rodent hookworm. A 2-step mechanism is proposed: antibody-induced metabolic damage that blocks feeding by the worms followed by worm expulsion by activated lymphocytes. Active infection initiates a far stronger response than is possible when either lymph node cells or serum is passively transferred. *Both* antibodies and cells are probably required for full expression of intestinal resistance, and the effect is synergistic rather than additive.

Trichinella infection sometimes presents characteristic clinical symptoms such as edema of the eyelids and face but often presents less specific clinical signs such as eosinophilia, which can be suggestive of several other parasitic infections. Specific immunodiagnostic tests may thus be of great importance. The bentonite flocculation test for human trichinosis is of value because of its high degree of specificity. In addition, there are many other immunodiagnostic tests, including complement fixation, hemagglutination, flocculation, immunofluorescence, soluble antigen fluorescent antibody, and a skin test (Bachman intradermal test). Both immediate and delayed responses are seen; the former shows that reaginic IgE is also produced.

In humans, infection with *Trichinella* initially elicits IgM antibody followed by an IgG response. IgA antibody has been reported, which is not surprising because the female worms are in the intestinal mucosa, though the locally produced protective gut antibodies probably are IgG1 rather than IgA or IgM, based on the *Nippostrongylus* studies. This antimetabolite reaction against the feeding worms is complement-independent and, as noted, precedes the rapid expulsion of the antibody-damaged worms by T lymphocytes. The precise mechanism by which these cells act—or their necessary association with other cells such as activated macrophages—remains a matter of dispute.

Although *Trichinella* is extremely immunogenic in its hosts, it can also exert an immunosuppressive action. Certain viral infections are more severe during infection with this parasite, and skin grafts show delayed rejection. On the other hand, cellular immunity to BCG seems to be potentiated when *Trichinella spiralis* is present, and *T spiralis*-infected mice are less susceptible to *Listeria* infections. These seeming contradictions may be explained by recalling that metazoan parasites contain a large complement of antigens, some of which may produce immunosuppression and others potentiation.

2. ASCARIASIS

Major Immunologic Features

- Specific antibody detectable.
- Elevated IgE.
- Responses to unrelated antigens potentiated or suppressed during infection.

Ascaris, the giant roundworm of humans, is a lumen-dwelling parasite as an adult and causes little inconvenience to the host except in the heaviest infections, though even single adult worms may produce mechanical damage by entering the bile or pancreatic ducts or penetrating a weakened gut wall. For example, worm penetration through an amebiasis intestinal lesion produces peritonitis. Ingestion of eggs results in larvae that migrate through the intestinal wall to eventually reach the lung via the bloodstream. In a previously infected host, hypersensitivity reactions in the lung resulting from high levels of IgE can cause serious pneumonitis. Acute hypersensitivity to *Ascaris* antigens often develops in laboratory workers and makes it virtually impossible for them to continue working with the nematode.

Cases of sudden death in Nigeria have been ascribed to *Ascaris*-induced anaphylactic shock, part of what has been termed "a helminth anaphylactic syn-

drome" heretofore rarely diagnosed or recognized. Death probably resulted from release of a mast cell degranulator by the worms, since degranulated mast cells were found throughout the body tissues in these children, or from a reagin-*Ascaris* allergen interaction at the mast cell surface. Allergy to ascariasis may underlie many of the symptoms of *Ascaris* infection, including abdominal pain.

Antibodies to *Ascaris* are of no diagnostic or protective value, although they are formed during infection; however, hemagglutination tests can be of epidemiologic value.

3. *TOXOCARA* INFECTIONS

Toxocara canis, the dog ascarid, is now known to infect small children who ingest its eggs in dirt. *Toxocara* eggs produce a population of migrating larvae that are immobilized in the tissues of humans and consequently never produce worms in the intestinal tract. Visceral larva migrans is characterized by high peripheral eosinophilia and chronic granulomatous lesions associated with the migrating larvae; such larvae in the eyes of infected children have been confused with retinoblastoma and diagnosed only after enucleation of the affected eyeball. Immunodiagnostic tests for visceral larva migrans have therefore been eagerly sought. Initially, lack of specificity for *T canis* was a great problem, but specific immunodiagnostic methods have been developed that should allow prompt diagnosis, and a sensitive ELISA test for antibody to this parasite is in common use in the USA. Visceral larva migrans can also be caused by larvae of other nematodes such as the common ascarids of cats *(Toxocara mystax, Toxascaris leonina)* and also some members of the genus *Capillaria*, which migrate in human tissue but do not develop further. Dog and cat hookworms *(Ancylostoma brasiliense, Ancylostoma caninum, Ancylostoma ceylonicum)* produce a similar "lost larva" condition in which skin-invading larvae from pet-contaminated sandy soil tunnel into the skin, where they produce serpiginous, pruritic, tracklike lesions, a condition called **cutaneous larva migrans.**

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36 Oral & Dental Diseases

John S. Greenspan, BSc, BDS, PhD, FRCPath

The mouth is the portal of entry for a variety of antigens, including numerous microorganisms, into the alimentary and respiratory systems. Normally, these antigens do not cause disease and are flushed away with swallowed saliva into the distal parts of the alimentary tract. Continual desquamation of oral epithelium, toothbrushing, and other forms of mouth cleaning mechanically protect the mouth. Immunologic defense mechanisms, particularly IgA, probably prevent adherence of microorganisms to mucosal and tooth surfaces by aggregating them and possibly rendering them more susceptible to phagocytosis.

Several of the most important oral diseases, including caries, the common forms of gingival and periodontal disease, oral herpes simplex infections, candidal infections, and the oral manifestations of primary and secondary immunodeficiency (including AIDS), are due to an imbalance between oral organisms and the host response. This imbalance may be a hypersensitivity phenomenon or may be the result of immunologic deficiency. Alternatively—particularly in the case of dental caries and chronic inflammatory periodontal disease—specific pathogenic microorganisms may directly damage the tissues regardless of the status of the host response.

Another group of oral diseases in which immunologic factors have been implicated are those in which oral tissues are a target for autoimmune reactions. Manifestations may be confined to the mouth or may involve oral tissues as part of a systemic disease. Many are mucocutaneous diseases and several are rheumatoid diseases; others involve mainly the gastrointestinal tract. The role of tumor immune mechanisms in oral homeostasis and the part that defects in these mechanisms play in the etiology and pathogenesis of oral precancerous lesions and mucosal malignancy constitute a rapidly growing field of interest. Tumor immune mechanisms are probably important but must be considered in the context of other factors, including oncogenic viruses and chemical carcinogens.

LOCAL ORAL DISEASE INVOLVING IMMUNOLOGIC MECHANISMS

INFLAMMATORY PERIODONTAL DISEASES: GINGIVITIS & PERIODONTITIS (See Fig 36–1.)

Major Immunologic Features

- Bacterial dental plaque induces inflammation of tissues immediately surrounding the teeth.
- The local responses of the host are not effective in eliminating the bacteria, which continue to adhere to the tooth surfaces. Humoral and cellular immunity are both involved in these responses.
- Local responses include complement activation, infiltration of leukocytes, release of lysosomal enzymes and cytokines, and production of a serous gingival crevicular exudate.
- Inflammatory agents from the bacteria and immunopathologic reactions of the host result in gingivitis and periodontitis.

General Considerations

Inflammation of the supporting tissues of the teeth produces one of the most common groups of human diseases. Depending on its severity, the destructive process may involve both the gingiva (gingivitis) and the periodontal ligament and alveolar bone surrounding and supporting the teeth (periodontitis). Periodontitis may involve both the direct cytotoxic and proteolytic effects of dental plaque and the indirect pathologic consequences of the host's immune response to the continued presence of bacterial plaque microorganisms.

Dental plaque is a mass of bacteria that adheres tenaciously to the tooth surfaces. In gingivitis, the plaque generates inflammation of the gingival tissue without affecting the underlying periodontal ligament and bone. In periodontitis, attachment between the gingiva and the involved teeth is lost, subgingival bacterial plaque forms on the root surfaces, and bone loss is clinically apparent (Figs 36–2 and 36–3). Elimination of the plaque usually stops the inflammatory pro-

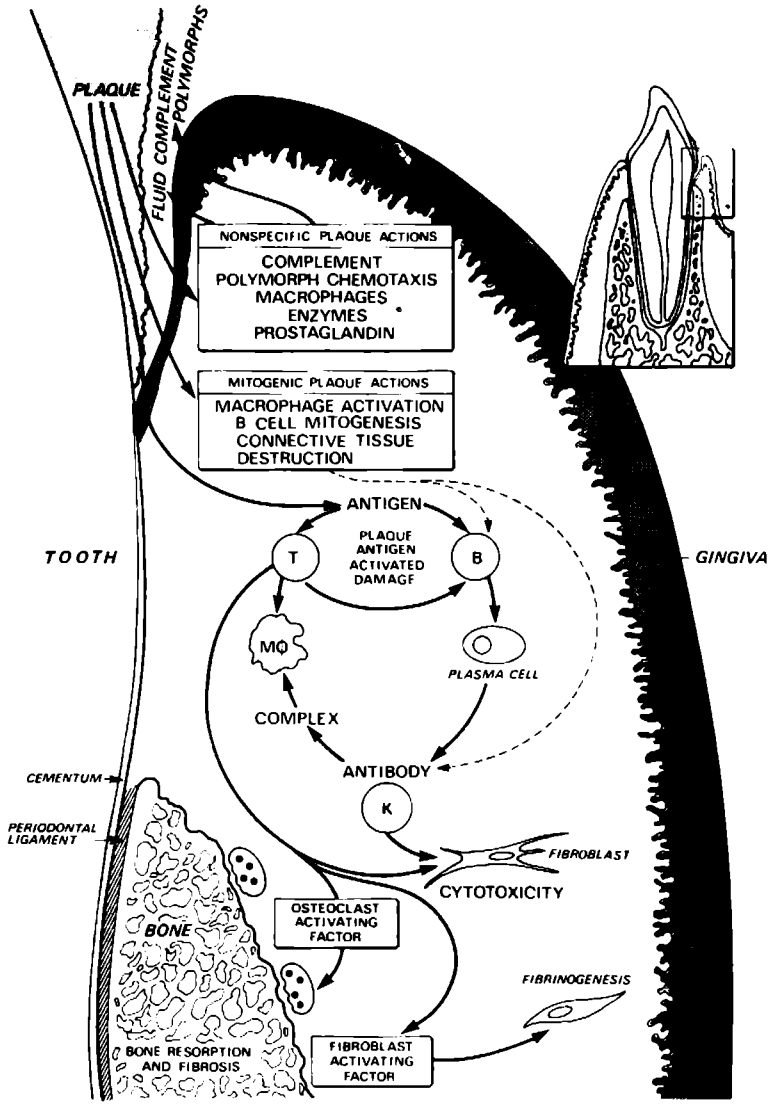

Figure 36–1. The pathogenesis of periodontal disease.

cess. In children with poor oral hygiene, gingivitis is common but periodontitis is rarely seen.

The microflora of the dental plaque is complex, comprising many different strains of bacteria (gram-positive rods and cocci and gram-negative rods, cocci, and filamentous forms). In general, the healthy gingival crevice contains only a few gram-positive streptococcal and facultative *Actinomyces* species. As gingivitis develops, many more gram-negative organisms are found, including *Fusobacterium nucleatum*, *Bacteroides melaninogenicus* ssp *intermedius*, and *Haemophilus* species. Many motile rods and spirochetes are also seen. In advanced adult periodontitis, the cultivable organisms consist predominantly of gram-negative anaerobic rods such as *Bacteroides gingivalis*, *B melaninogenicus* ssp *intermedius*, and *F nucleatum*. Furthermore, as many as 50% of organisms from such lesions are found to be motile rods and spirochetes on phase contrast examination. There is some indirect evidence for a relationship between particular forms of periodontal disease and specific microorganisms. Thus, elevated levels and increased frequency of serum antibodies to *Actinobacillus actinomycetemcomitans* are found in localized juvenile periodontitis (rapidly progressive periodontitis) (see below).

Immunologic Pathogenesis

A delicate balance exists between dental plaque organisms and the host response. In health, the immuno-

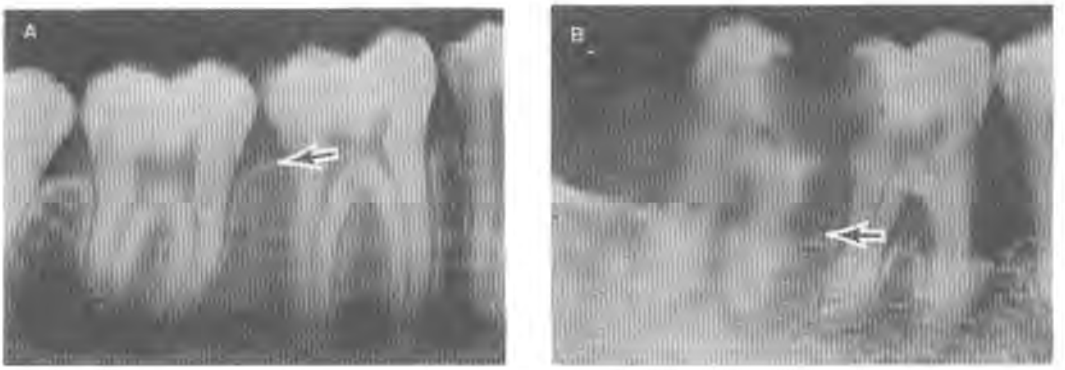

Figure 36–2. Radiographs of the lower molars of a 25-year-old man with a normal periodontium *(A)* and a 45-year-old man with advanced periodontitis and severe dental caries *(B)*. In the patient with periodontitis, more than half of the supporting alveolar bone has been destroyed (arrows). (Courtesy of GC Armitage.)

logic machinery provides a well-regulated specific defense against infiltration by plaque substances. The tissue-destructive mechanisms thought to be involved in periodontal disease include direct effects of plaque bacteria, PMN-induced damage, complement-mediated damage initiated by both antibody and the alternative pathway, and cell-mediated damage.

Clinically apparent gingivitis is probably the result of an exaggerated response to bacterial plaque. Individuals with mild gingivitis have, in addition to a continued polymorphonuclear infiltration, a gingival influx of a few lymphocytes believed to be T lymphocytes. However, those with prolonged severe gingivitis and severe periodontitis have an influx composed mainly of B lymphocytes and plasma cells, the latter committed mainly to IgG production. Most noteworthy in severe periodontal disease is the extremely low proportion of the gingival plasma cells committed to IgG2 production, while serum levels of the IgG subclasses are normal. The proportions of IgG3, IgG1, or IgG4 in the gingival tissues with specific antibody activity for plaque antigens are unknown. The unusual local IgG subclass response may indicate a degree of nonspecific activation of B lymphocytes arriving in the inflamed area, possibly caused by a variety of mechanisms involving bacterial mitogens and proteases and continued activation of the alternative complement pathway. Associated with gingivitis is the generation of a serum exudate known as crevicular fluid, which flows from around the teeth and contacts the dental plaque. This exudate, like serum, contains functional complement components as well as low levels of specific antibodies to the various plaque antigens.

The onset of flow of serous crevicular fluid is an important stage in the progression of periodontal disease. Crevicular fluid complement is rapidly activated by a combination of effects. These include activation of the classic pathway by IgG and IgM antibodies to subgingival plaque antigens; activation of the alternative complement pathway by endotoxins and peptidoglycan from gram-negative and gram-positive mi-

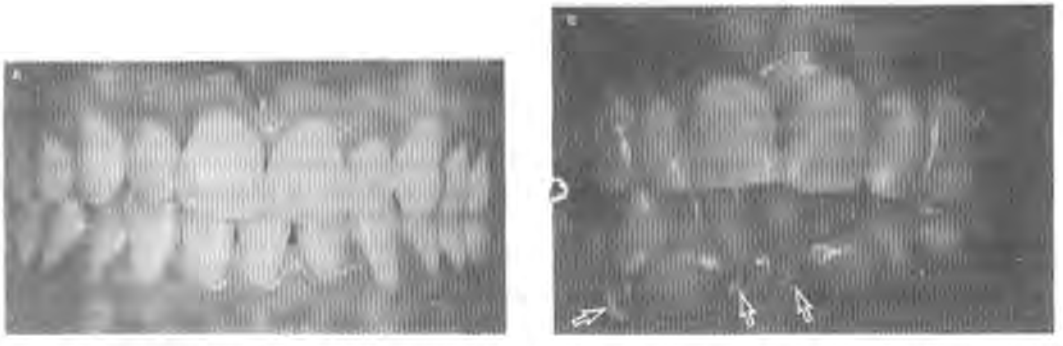

Figure 36–3. Clinical appearance of the anterior teeth and periodontal tissues of a 22-year-old man with healthy gingiva *(A)* and a 48-year-old man with advanced periodontitis *(B)*. In the patient with periodontitis, note the heavy deposits of plaque and calculus (arrows). Marked gingival inflammation is particularly noticeable around the lower anterior teeth. Most teeth have either pocket formation or extensive gingival recession. (Courtesy of GC Armitage.)

croorganisms, respectively; and activation of complement components by host and bacterial proteolytic enzymes. Complement activation results first in release of C3a and C5a, which causes additional edema and increases crevicular fluid flow, and subsequently in chemotactic attraction of polymorphonuclear leukocytes. Other chemotactic factors are produced directly by the plaque microorganisms. The release of proteolytic enzymes (eg, collagenaselike and trypsinlike activities) by host cells is believed to damage tissue and activate additional complement components. Complement activation also results in damage to bystander cells by terminal complement components and subsequent release of prostaglandin E. In vitro, prostaglandin E can induce bone resorption through its effect on osteoclasts, but the mechanism of its action is unknown.

Cell-mediated immunity may also play a role in the progression of periodontal disease. In some studies, individuals with periodontal disease generally exhibit increased peripheral blood T lymphocyte reactivity to plaque antigens. Yet, for reasons unknown, in severe gingivitis and severe periodontitis, the local T cell response to the plaque is conspicuously small.

Bone destruction in periodontal disease may be mediated by lymphokines, including osteoclast-activating factor, as well as by parathyroid hormone and agents such as prostaglandins.

Individuals with reduced immunologic capacity, both primary immunodeficiency and immunodeficiency secondary to treatment associated with kidney transplantation, do not have more gingival and periodontal disease than normal controls. Indeed, in some studies, immunodeficient patients had better gingival health and lower caries experience than their healthy controls.

The rate of the healing process is closely coupled to immunologic responses. In fact, a limited action of proteolytic enzymes can induce, through a complicated sequence, partial regeneration of destroyed host tissues including bone. Some individuals, however, appear to be unable either to specifically control the plaque bacteria or to establish a regulated controlled response to its continued presence. Together with the proteolytic and cytotoxic activity of the plaque, the poorly directed immunopathologic response causes disruption and apical migration of the epithelial gingival attachment to the tooth and thus creates a larger surface area between the bacterial plaque and the host's tissue. The pathologic cycle continues, as immune responses alone are apparently unable to destroy and remove subgingival bacteria.

Clinical Features

In gingivitis, there is generally an accumulation of dental plaque on the tooth adjacent to the inflamed gingiva, which is dark red, especially at the gingival margin. Edematous swelling masks the normal stippled appearance seen in healthy gingiva, and bleeding often occurs, especially after toothbrushing or probing. The flow rate of the crevicular exudate is related to the disease severity. In chronic gingivitis, gingival fibrosis may occur, and the inflammatory features may be more difficult to assess.

Chronic inflammatory periodontal disease follows many cases of gingivitis. (Compare Figs 36–2 and 36–3.) This condition is characterized by progressive inflammatory destruction of periodontal ligament and alveolar bone, apical migration of the epithelial attachment, and pocket formation due to detachment of gingiva from tooth. In severe chronic inflammatory periodontal disease where substantial alveolar bone loss has occurred, the teeth become mobile; if the disease progresses, the involved teeth are lost.

Immunologic Diagnosis

Lymphocytes from individuals with periodontal disease are more responsive to dental plaque antigens, but no clear relationships have been found between disease severity and serum or salivary antibody levels. At present, periodontists generally do not use any immunologic test in the diagnosis of gingivitis and periodontitis. Most individuals with inflammatory periodontitis have gingivitis, but the clinical symptoms of the latter may be masked by fibrosis.

Treatment & Prognosis

Although gingivitis and periodontitis are apparently caused by dental bacterial plaque, there is a reluctance to treat this disease with antibiotics, because elimination of one group of organisms by antibiotics may lead to the emergence of antibiotic-resistant strains of organisms. Depending on the severity of the periodontal disease, treatment may range from simply good routine oral hygiene to periodontal surgery. Reducing plaque accumulation to an absolute minimum is essential for the arrest of gingivitis or the reduction of periodontal ligament destruction and bone loss.

JUVENILE PERIODONTITIS

In a small percentage of the population, periodontal bone loss occurs very rapidly, sometimes within 2–5 years. In this condition—juvenile periodontitis, formerly known as periodontosis—conventional periodontal treatment is ineffective. A characteristic gram-negative anaerobic flora, different from that in the more slowly progressive form of periodontitis, is found. Some periodontists find that short-term antibiotic therapy is useful in these cases, but there is no evidence that the results of such treatment would be permanent. Several reports suggest that defects in granulocyte or monocyte function may be involved.

PHENYTOIN-INDUCED GINGIVAL HYPERPLASIA

Individuals who receive phenytoin (Dilantin) often develop severe gingival hyperplasia in response to accumulations of dental plaque, perhaps as a result of an

extraordinary local response by fibroblasts and epithelial cells. This response may be due to the effects of phenytoin on fibroblasts and epithelial cells and on the immune system. For example, phenytoin affects salivary and serum immunoglobulin levels and may induce changes in the salivary glands. Induced IgA deficiency has been noted in some individuals who have received phenytoin since infancy.

ACUTE NECROTIZING ULCERATIVE GINGIVITIS

Acute necrotizing ulcerative gingivitis is a severe form of gingivitis most commonly seen in adolescents and young adults. The onset is sudden, with pain, gingival bleeding, severe halitosis, and destructive lesions that are most severe in the interdental papillary region. There may be systemic features, including lymphadenopathy, fever, and malaise. The lesions may spread and may also involve other parts of the oral mucous membrane.

Microscopy shows gingival epithelial necrosis with ulceration, a mixed inflammatory cell infiltrate, destruction of the connective tissue in the superficial part of the lesion, and underlying vasodilatation and vascular thrombosis.

A mixed bacterial flora is present that resembles the normal oral flora. There is evidence that spirochetes invade the tissue and penetrate the connective tissue. There have been few immunologic studies. The only significant finding is increased in vitro lymphocyte activation to certain plaque organisms in patients with this disease as compared with healthy controls. However, the results are very similar to those found in chronic inflammatory periodontal disease and may represent a result rather than the cause of the condition. The lack of significant increases in antibody titers to putative causative organisms during the recovery phase of the condition is evidence against primary infection by one of these organisms.

DENTAL CARIES

Dental caries is a bacterial disease in which the hard structures of the teeth are progressively destroyed by a process involving both the mineral and organic components of enamel and dentin. The principal causative organism of enamel caries is *Streptococcus mutans*, although other organisms may play a role. *S mutans* produces acids that are capable of dissolving enamel. Another important factor is production of a favorable environment through accumulation of bacterial plaque on the tooth surface, partly due to *S mutans* itself, which is capable of manufacturing the dextran bulk of the plaque with its enzyme glucosyltransferase.

One means of caries prevention has involved increasing the resistance of tooth structures through the use of fluoride, either as a public health measure in drinking water or as a topical agent in individual patients. Additional prophylactic measures include attempts to prevent the accumulation of bacterial plaque by measures designed to improve hygiene, by the use of antibacterial agents, and by restriction of fermentable carbohydrates in the diet. Only recently has an immunologic approach been applied to the problem of caries prevention, although the conceptual basis for this approach has been available for over 50 years. The delay was due partly to the difficulty in identifying the causative organisms among the multitude of normal oral flora; partly to the limited availability of acceptable animal models for caries; and partly to the reluctance of many workers to accept the notion that the hard, nonvital enamel surface, which is "outside the body," could be protected by immunologic means. With the establishment of *S mutans* as the probable causative organism and the realization that the gingival sulcus fluid continually brings high concentrations of antibodies to the enamel surface, interest in immunization against dental caries has intensified. Attempts to immunize rodents and monkeys against *S mutans* whole cells or various forms of antigen preparations, including glucosyltransferase, have produced promising results. Significant levels of anti-*S mutans* serum or salivary antibodies and decreased or delayed inception of carious lesions have been reported. Concern has been expressed that antibody to *S mutans* might cross-react with host tissues and possibly produce rheumatic fever, although it is not clear that the bacterial antigen eliciting the anti-caries immune response is also capable of inducing the harmful autoimmune process.

RECURRENT ORAL ULCERATION (Aphthous Stomatitis)

Major Immunologic Features

- Lymphocyte infiltration present at earliest stage of the lesion.
- Circulating antibodies to oral mucous membrane present in some patients, which may cross-react with oral organisms.
- Cellular immunity to the same antigens reported.
- Circulating immune complexes found in some patients.
- Association with HLA antigens.
- Favorable response to topical or systemic corticosteroids.

General Considerations

After caries and chronic periodontal disease, oral ulceration probably represents the most common lesion of the mouth. Although oral ulcers can be due to a large number of diseases, the most common form is recurrent oral ulceration (aphthous stomatitis). Recurrent oral ulcers usually occur alone but may be a local manifestation of Behçet's syndrome, along with uveitis or genital ulcers and perhaps lesions of other sys-

tems. Estimates of the incidence of recurrent oral ulceration vary, but 20% is probably reasonable. The condition may recur only once or twice a year or may be so frequent that a new set of ulcers overlaps a previous group. There is slight evidence of a familial incidence. Emotional and nutritional factors may play a causative role, and an association has been suggested with changes in the hormone status during the menstrual cycle. Extensive searches for specific bacterial or viral causes have been unsuccessful. A possible role for herpes simplex virus (HSV) type 1 has again been raised by the observation that part of the HSV genome is present and transcribed in peripheral blood mononuclear cells of patients with recurrent aphthae and Behçet's syndrome. Evidence also indicates a role for *Streptococcus sanguis*, since this organism has been cultured from the ulcers and patients exhibit delayed hypersensitivity reactions to the organism and significant inhibition of leukocyte migration in vitro. However, the organism is a common commensal. Furthermore, another study has shown reduced lymphocyte transformation to the organism in patients as compared with controls. A more likely role for bacterial or viral agents in this disease is that of cross-reacting antigens eliciting host responses to autologous oral mucous membrane antigens.

Immunologic Pathogenesis

Patients have a raised level of circulating antibody to a saline extract of fetal oral mucous membrane. Slightly raised levels of the same antibody have been found in other ulcerative conditions but at lower titers. The antibodies are of the agglutinating and complement-fixing types, suggesting that antibody cytotoxicity might be involved in the tissue destruction. However, other studies show poor correlation between the level of anti-mucous membrane antibody and clinical features of the disease. In addition, 2 other mechanisms could explain the presence of circulating autoantibodies of this type. The antibodies may represent a cross-reaction between antigens of an organism present in the mouth, such as *S sanguis* or a virus, and oral mucous membrane epithelial cells. Alternatively, the antibodies may be produced because ulceration has repeatedly exposed tissue antigens that had previously been protected from the immune system. There have been unsuccessful attempts to show that patients' serum containing significant titers of this antibody has a direct cytotoxic effect against oral epithelial cells. Thus, it is unlikely that the direct action of a cytotoxic anti-oral mucosal antibody is involved in the pathogenesis.

Interest in the role of cellular immune mechanisms in the pathogenesis of recurrent oral ulceration was aroused by the observation that the earliest histologic changes involve the presence of an infiltrate of lymphocytes. Other cells do not appear until a later stage. Furthermore, patients with recurrent oral ulceration were shown to have peripheral blood lymphocytes that were sensitized to oral mucous membrane antigen. These 2 observations support the hypothesis that a cell-mediated hypersensitivity mechanism might be involved in the pathogenesis of the lesion. Lymphocytes from some patients with recurrent oral ulceration are cytotoxic to oral epithelial cells. The antigen eliciting the cytotoxic reaction has not been identified. It might be one or more epithelial cell surface autoantigens, determinants cross-reacting with an infecting organism or organisms, or food or microbial antigens attached to oral epithelial cell surfaces or even produced by a hapten mechanism. Increased antibody-dependent cellular cytotoxicity has been found. The identity of the population of lymphocytes involved in these reactions is also unknown. There is at present no acceptable hypothesis linking oral mucous membrane autoantigen and effector mechanisms, although transient defects in immunoregulation have been postulated.

Patients with Behçet's syndrome and recurrent oral ulceration show elevated levels of serum C9 and of circulating soluble immune complexes. IgG and C3 have also been demonstrated in the basement membrane zone of the lesions. It is not clear whether these observations offer further clues to the immunologic pathogenesis of the disease or represent epiphenomena. There is also some evidence for an increased incidence of HLA-B12 in recurrent oral ulceration.

Clinical Features

Recurrent oral ulceration is a common disease characterized by painful recurrent necrotizing ulcerations of the nonkeratinized oral mucous membrane. The ulcers are covered with a gray membrane surrounded by an erythematous ring. Ulcers may form singly or in groups and may appear infrequently or be so common that the patient is never free from disease. The current classification recognizes 3 subtypes. Major aphthae are large (> 5 mm in diameter) and are often long-lasting. Minor aphthae (< 5 mm in diameter) are the common form. A rarer subtype known as herpetiform ulceration is characterized by clusters of very small ulcers. Herpetiform ulceration, which bears no relationship to herpesvirus lesions, may represent a different disease.

At the very early stage, prior to fresh ulceration, when the patient first becomes aware of a change in sensation or a small itchy nodule, the microscopic appearance is that of a lymphocytic infiltrate. Once ulceration has occurred, the predominant appearance is that of acute inflammation, with vascular dilatation, polymorphonuclear leukocyte and fluid exudation, and surface necrosis. There may be chronic inflammation, with lymphocytes and plasma cells in the deeper part of the lesion. The smaller ulcers heal at about 4 days, and no scar is produced. Major aphthous ulcers may last several weeks or months, and such lesions extend deeper into the underlying connective tissue, minor salivary glands, and muscle and then heal with scarring. The ulcers may interfere with speech, mastication, and swallowing because of pain and their location. In such cases, the patient may be unable to work and may lose weight.

Differential Diagnosis

The diagnosis is often based on the history of recurrent ulceration in the mouth and on the clinical appearance. The disease must be distinguished from traumatic ulceration, primary herpetic gingivostomatitis, recurrent labial herpes, the oral lesions of vesiculobullous diseases such as pemphigus, pemphigoid, erythema multiforme, and erosive lichen planus, and oral ulcerations associated with ulcerative colitis. Crohn's disease, celiac disease, other malabsorption states, and hematologic abnormalities must also be considered. The last subject is controversial, but several studies indicate a significant incidence of hematologic and malabsorption problems in patients presenting with clinical recurrent oral ulceration. The underlying diseases discovered during these studies include gastric carcinoma, celiac disease, and a variety of inflammatory bowel diseases. The oral ulcers usually resolved when the underlying condition was treated, but they did not respond to local measures. If, as is suggested by some studies, as many as 20% of cases are due to underlying, possibly serious systemic disease, extensive investigation would be indicated, including screening of all patients by means of a complete blood count and determinations of serum iron, vitamin B_{12}, and folic acid.

The oral ulcers of Behçet's syndrome are identical to those in other forms of disease, but the syndrome can usually be diagnosed because of the presence of ocular lesions or genital ulceration or because of the involvement of other systems. Oral ulceration can be a feature of neutropenia or agranulocytosis associated with marrow defects caused by drugs or other factors. The oral ulcers of cyclic neutropenia are very similar and may occur in the absence of other symptoms.

Treatment & Prognosis

Effective treatment depends on identification of any underlying systemic disease. In such cases, treatment of the systemic condition usually leads to cure of the oral ulceration. For the remaining group, uncomplicated by known systemic disease, several treatment forms are available. They involve the use of topical corticosteroids, antibiotics, and immunostimulants. The most effective topical corticosteroids available are 0.1% triamcinolone in Orabase, 2.5-mg tablets of hydrocortisone sodium succinate, and 0.025% fluocinonide in Orabase. Some cases of major aphthous ulceration are sufficiently severe to warrant the use of systemic prednisone. Tetracycline mouth rinses have been used with some success in the herpetiform variety of recurrent oral ulceration. The treatment of Behçet's syndrome is dealt with in Chapter 21.

SYSTEMIC DISEASES WITH ORAL MANIFESTATIONS INVOLVING IMMUNOLOGIC MECHANISMS

The causes of the immunologic diseases discussed below that affect oral mucosa have not been determined. However, several recent findings in animals in which autoantibodies have been experimentally induced suggest an association of these diseases with (1) the continued presence of damaged or chemically modified host tissue and (2) genetic factors which affect T lymphocyte suppressor, helper, or recognition functions. Deposition of autoantibody on its host antigen will activate the classic complement pathway. In turn, this will activate the alternative complement pathway through C3b and initiate complement-dependent tissue destruction.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

The oral mucosa is particularly hospitable to opportunistic pathogens. Thus, primary and recurrent herpes simplex, varicella-zoster virus, and several fungi, notably *Candida* species, are frequent features of primary immunodeficiency syndromes (see Chapter 20). The same conditions, as well as a number of others, are seen in patients whose immune systems are compromised by chemotherapy, those receiving bone marrow transplants, patients with leukemia or lymphoma, and those with clinical expressions of AIDS retrovirus (HTLV-III/LAV/ARV)-induced immunosuppression.

The oral features of AIDS include Kaposi's sarcoma, non-Hodgkin lymphoma, and severe oral candidiasis as well as persistent herpesvirus lesions (HSV and VZV). Other conditions seen in AIDS and AIDS-related complex (ARC) and in patients with less severe forms of AIDS retrovirus-induced immunosuppression include severe periodontal disease, oral warts, and the recently described lesion **oral hairy leukoplakia.**

Hairy leukoplakia is seen on the tongue in immunosuppressed male homosexuals. Ninety-nine percent of patients are AIDS-virus antibody-positive, and a majority of those tested carry the virus in blood lymphocytes.

The lesion has characteristic histopathologic features suggestive of human papillomavirus (HPV), further evidence for the presence of which is provided by antigen staining and electron microscopic morphology. However, no HPV-DNA is found by hybridization techniques. In fact, clear evidence for the presence of Epstein-Barr virus (EBV) comes from immunocytochemistry using monoclonal antibodies, from electron microscopic morphology, and from

DNA studies with EBV probes. Southern blot electrophoresis provides evidence for the presence of EBV-DNA in complete linear virion form and in very high copy number. About 20% of patients with hairy leukoplakia have AIDS at the time of diagnosis of hairy leukoplakia, but a very high number of those who are AIDS-free when first seen subsequently develop AIDS, with mean conversion rates of 48% at 16 months and 83% at 30 months, mostly with *Pneumocystis carinii* pneumonia.

Oral hairy leukoplakia is clearly a significant indicator of AIDS virus-induced immunosuppression and is highly predictive of the subsequent development of AIDS. It appears to be one of only 2 lesions uniquely associated with AIDS virus infection. It is the first form of oral leukoplakia consistently associated with a virus or viruses.

The mechanism whereby the AIDS retrovirus favors oral opportunistic infection presumably involves viral elimination of helper T cells and thus loss of cell-mediated immunity to herpes-group viruses and fungi as well as to other organisms. However, other mechanisms may also mediate the immune defect, including loss of Langerhans cells or their functions, as well as polymorphonuclear and macrophage aberrations.

PEMPHIGUS

Pemphigus is a vesiculobullous disease in which autoantibody to host epithelial intercellular substance is produced (Fig 36–4) (see Chapter 29). No information is available about the cause and little about the nature of the cell surface glycoprotein antigen. Both the mucosal and the skin blisters contain free-floating epithelial cells coated with antibody and complement. The mechanism of acantholysis involves activation of a proteinase, possibly plasminogen activator. It is not known why lesions commonly appear first on the oral mucosa. However, prompt treatment with systemic corticosteroids may prevent spread of the disease to skin. Direct immunofluorescence should be a routine procedure in the diagnosis of oral vesiculoerosive disease.

BENIGN MUCOUS MEMBRANE PEMPHIGOID

Benign mucous membrane (cicatricial) pemphigoid is a rare chronic blistering, scarring autoimmune disease with a predilection for mucosal surfaces (see Chapter 29). The recently defined linear IgA disease may present with similar lesions. The host produces autoantibody (IgG, IgA, or IgM) against basement membrane zone substances (Fig 36–5). Subsequent complement binding has also been demonstrated in the majority of cases. This probably leads to release of anaphylatoxins that activate mast cells to release eosinophil chemotactic factor. Eosinophils then release enzymes and reactive oxygen intermediates in the basement membrane zone, causing destruction and blister formation. The original antigenic stimulus is not known, although evidence suggests that the disruption occurs at the site of interaction of laminin with type IV collagen. Circulating antibodies against host basement membrane antigens have been demonstrated in a number of individuals with this disease.

The condition known as **desquamative gingivitis** can be a manifestation of pemphigus, pemphigoid, erythema multiforme, or lichen planus.

The diagnosis of oral vesiculobullous disease involves biopsy and direct immunofluorescence as well as indirect immunofluorescence with the patient's

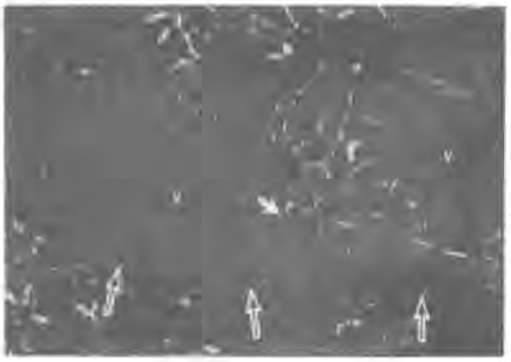

Figure 36–4. Direct immunofluorescence on buccal mucosa from a patient with pemphigus vulgaris. The conjugated antihuman IgG is bound in the intercellular spaces of the epithelial cells and to the surface of the cells undergoing acantholysis (solid arrow). Intraepithelial vesicles (V) form above the row of basal epithelial cells attached to the basement membrane (open arrows). Original magnification × 312. (Courtesy of TE Daniels.)

Figure 36–5. Direct immunofluorescence on gingiva from a patient with mucous membrane pemphigoid. The conjugated antihuman C3 forms a continuous linear fluorescent pattern between the epithelium (E) and connective tissue (CT) in the basement membrane zone. Original magnification × 156. (Courtesy of TE Daniels.)

serum. Systemic corticosteroids are used in the treatment of oral pemphigoid.

CHRONIC DISCOID LUPUS ERYTHEMATOSUS

Chronic discoid lupus erythematosus is a disease of unknown cause characterized by skin and oral mucosal lesions that are clinically similar to the chronic scarring lesions seen in 10–20% of patients with systemic lupus erythematosus (SLE). Typical histologic changes in oral mucosal lesions consist of hyperkeratosis or atrophy, hydropic degeneration of basal cells, and perivascular lymphocytic infiltrates in the connective tissue. Unlike patients with SLE (see Chapters 21 and 29), patients with chronic discoid lupus erythematosus rarely have any systemic manifestations; however, about 5% of the patients ultimately develop SLE. In chronic discoid lupus erythematosus, deposition of immunoglobulin and complement usually occurs only in the areas of the visible lesion, whereas in SLE deposition occurs even in nonlesional mucosa and skin. In both diseases, a granular deposition of immunoglobulin and complement can occur at the mucosal-submucosal interface or in skin at the dermal-epidermal junction. Significant levels of antinuclear (anti-DNA) antibodies are not found as they are in SLE.

ERYTHEMA MULTIFORME

Erythema multiforme is an acute inflammatory disease the manifestations of which range from a few inflamed cutaneous and mucosal lesions to a rare multisystem and sometimes fatal disorder. The prodromal and inflammatory stages are usually short and the initial erythema usually fades in 3–7 days, leaving hemorrhagic papules. Polymorphous skin lesions form annular or figured patterns, and bullous erosive involvement of the mucous membranes often occurs.

The cause is unknown, but infection, including HSV, and a number of drugs and environmental factors have been suggested as possible causes. Erythema multiforme may be a form of contact dermatitis, though confirmatory evidence is not available. Efforts to identify specific microbial agents have been unsuccessful. Perivascular C3 and IgG have been found in the lesions. If the immune system is involved in the pathogenesis, it is probably at the prodromal stage. Treatment with corticosteroids or other immunosuppressants is not routinely indicated.

LICHEN PLANUS

This relatively common inflammatory disease of the skin often includes oral features, and about one-third of cases of oral lichen planus are associated with skin lesions. The oral lesions vary in character from flat white areas resembling hyperplastic or dysplastic lesions to extensive areas of erosion or ulceration. The latter can be extremely troublesome. Histologically, the lesion is characterized by a tendency to epithelial thickening, with hyperplasia and degenerative changes in the basal area of epithelium. The underlying connective tissue shows a dense infiltrate of round cells that are mostly lymphocytes and macrophages. Several studies have shown that these lymphocytes are predominantly (even exclusively) T cells, mostly of the suppressor/cytotoxic phenotype. The pathogenic mechanism involved in the production of the lesions is unknown. Possibilities include microbial agents or antigens (either bacterial or viral), hypersensitivity to mercury in amalgam fillings, and autoimmunity. Some reports suggest the presence of a lichen planus-specific epidermal antigen in cutaneous lichen planus. Indirect immunofluorescence does not reveal any characteristic immunoglobulin deposition, but there is a consistent pattern with binding of antiserum to fibrinogen in the basement membrane and superficial connective tissue zone. Attempts to demonstrate cell-mediated immunologic defects have been disappointing. Lesions similar to those of lichen planus can be seen in graft-versus-host disease. The oral lesions of the erosive variety respond to topical corticosteroids, but these agents do not cure the disease. There is a potential for malignant transformation in lichen planus, and caution must therefore be exercised in long-term use of corticosteroids in this disease.

INFLAMMATORY BOWEL DISEASES WITH ORAL MANIFESTATIONS

Ulcerative colitis and Crohn's disease (see Chapter 25), which classically involve the intestinal mucosa, may also involve the mouth. In ulcerative colitis and, to a much lesser extent, in Crohn's disease, the host lymphocytes may be cytotoxic for colonic epithelial cells. This may be a primary defect or may be due to low levels of suppressor cell activity, although no abnormalities in peripheral blood T cell subpopulations have been found. Moreover, in a low percentage of cases, high titers of circulating autoantibodies are detected against colonic mucosal epithelial mucopolysaccharides. These antibodies react with bacterial lipopolysaccharide antigens (*Escherichia coli* O14), and bacteria might be partially responsible for the autoantibody response in genetically susceptible individuals. In both diseases, the intestinal mucosa is damaged; subsequently, one might expect a higher penetration of bacterial antigens and, at the onset of the disease, an elevation of the mucosal antibody response (serum and secretory IgA).

The cause of the oral mucosal lesions is not known. Damage to intestinal mucosa may lead to alteration of mucosal cell structures so that they too may become antigenic. Patients with Crohn's disease have serum antibodies that react with their own oral mucosa in an immunofluorescence system.

Crohn's disease can involve the oral mucosa and lips, either in association with the gastrointestinal features of the disease or as its sole manifestation. There may be ulcers of the aphthous type, linear ulcers, raised nodules producing a "cobblestone" appearance on the buccal mucosa, or ridges resembling the fibroepithelial hyperplasia induced by ill-fitting dentures. Lips may show marked enlargement and cracking. Biopsy reveals multiple noncaseating granulomas with no evidence of acid-fast organisms. An increase in the incidence and severity of dental caries has been reported. When the oral features appear alone, local topical corticosteroids may be effective.

SARCOIDOSIS

Sarcoidosis (see Chapter 26) is a granulomatous disorder of unknown cause that most commonly affects middle-aged individuals. Many organs and tissues may become involved, particularly the intrathoracic tissues, hilar and paratracheal lymph nodes, and lung parenchyma. In a minority of cases the peripheral lymph nodes, eyes, skin, spleen, nervous system, and salivary glands may be affected. There is cutaneous anergy, and aberrations in peripheral blood lymphocyte populations have been described.

About 6% of individuals with sarcoidosis have salivary gland involvement. Swelling of major or minor salivary glands and xerostomia can occur. Labial salivary gland biopsy may be useful in excluding other causes of these signs. Oral mucosal lesions may be seen and include red or brown patches that are firm and painless or may ulcerate.

SJÖGREN'S SYNDROME

Sjögren's syndrome is a chronic inflammatory disease with widespread manifestations. The major features involve the salivary and lacrimal glands, and one of the connective tissue diseases is often present. Sjögren's syndrome can be diagnosed with certainty when 2 out of these 3 components are present. In addition to the dry mouth and dry eyes, patients frequently exhibit dryness of the upper respiratory tract, the ears, and the vaginal mucosa (see Chapter 21).

Oral Signs & Symptoms

Patients may complain of dryness of the mouth, soreness of the oral mucous membranes, otherwise inexplicable difficulties in wearing dentures, changes in taste sensation, or dysphagia. There may be a history of salivary gland swelling, but this is not necessary for the diagnosis. The xerostomia frequently leads to extensive dental caries, with numerous lesions at the cervical margins of the buccal surfaces and on the incisal and smooth occlusal surfaces. The latter finding is pathognomonic of xerostomia.

Heretofore, assessment of xerostomia has usually been subjective, but there are now well-established objective criteria. These include measurement of stimulated parotid flow rate under standardized conditions and semiquantitative assessment of chronic inflammatory involvement of the minor salivary glands of the lip. Labial salivary gland biopsy is a readily performed procedure that has revolutionized the diagnosis of this disease. Interdisciplinary cooperation between rheumatologists, ophthalmologists, stomatologists, and immunologists is essential for the proper diagnosis and management of Sjögren's syndrome.

The differential diagnosis includes all other causes of chronic xerostomia and salivary gland swelling, ie, drug-induced xerostomia, irradiation xerostomia, diabetes, tuberculosis or sarcoidosis, and salivary gland involvement in lymphoproliferative disorders. Primary salivary gland neoplasms usually develop in one gland, whereas the swelling in Sjögren's syndrome is frequently, but not always, bilateral. Other causes of salivary gland swelling include acute infection, salivary gland duct stones, mumps and cytomegalovirus infection, cirrhosis, and hypolipoproteinemia.

Pathogenesis & Features of the Salivary Gland Lesion

The characteristic lesion of Sjögren's syndrome in the major salivary glands is an infiltrate of lymphocytes, plasma cells, and macrophages that progressively replaces the acinar cells. Ducts may remain, and islands of epithelial cells, presumably proliferating from these duct remnants, may stand out against a monotonous background of chronic inflammatory cells. Although these islands are often referred to as "epimyoepithelial islands," there is no evidence that myoepithelial cells contribute to them. The progressive replacement of secretory acinar tissue causes the functional failure of the salivary glands and presumably also causes the swelling of the major gland when it occurs. The counterpart of this lesion in the minor salivary glands is focal sialadenitis, again with replacement of acini and survival of ducts. However, in the minor salivary glands, epimyoepithelial islands are rarely seen.

Some workers have described as a separate entity a histologically identical lesion of the major glands, which they term benign lymphoepithelial lesions. However, most if not all cases of this entity represent examples of Sjögren's syndrome. The benign lymphoepithelial lesion originally described by Mikulicz and identified for many years as Mikulicz's disease probably also is Sjögren's syndrome. Unfortunately, the situation was further confused by the use of the term Mikulicz's syndrome for swelling of major salivary glands due to other causes, including tuberculosis, diabetes, lymphoma, and leukemia.

The salivary gland lesion of Sjögren's syndrome and the benign lymphoepithelial lesion (Mikulicz's disease), which probably represents a variant, are associated with a tendency to develop malignant lymphoma. The risk of transformation into non-Hodgkin's lymphoma has been estimated to be 44

times that of the general population. There also appears to be an increased risk of skin cancer. Sometimes the lymphoma develops within the salivary gland lesion, but more often it occurs elsewhere. An intermediate stage (pseudolymphoma), which may represent a form of immunoblastic lymphadenopathy, has also been described.

The mechanism causing the progressive accumulation of lymphocytes, plasma cells, and macrophages within the salivary glands is unknown. The lymphocytes appear to be of both B and T cell types, with the majority being of helper phenotype. The mechanism of acinar cell destruction is also unknown, although direct cellular cytotoxicity, natural killer cytotoxicity, or an antibody-dependent cellular cytotoxic mechanism seems likely. These autodestructive mechanisms may be induced by viral antigens, virally modified autoantigens, or abnormal autoantigens of other types. Conversely, a primary abnormality in the control of lymphocyte autoreactivity might be present because of a regulatory defect involving suppressor or helper T cell abnormalities.

Although autoantibodies to salivary duct epithelium have been described in some cases of Sjögren's syndrome and rheumatoid arthritis, these do not appear to be cytotoxic and are probably not a factor in the pathogenesis of the salivary gland lesion. There is some evidence that the very earliest lesion consists of a perivascular lymphomonocytic infiltrate, but vasculitis is not seen and immune complexes have not been reported. No ready explanation exists for the higher incidence of Sjögren's syndrome in women.

Treatment

The treatment of the oral manifestations of Sjögren's syndrome is directed toward symptomatic relief of oral dryness and prevention of dental caries. While salivation may be stimulated by sour drinks or sour candies, sugar-containing or acid preparations must be rigorously avoided. Many patients consume large quantities of water or other liquids, but urinary frequency or nocturia often results. Mouth rinses containing methylcellulose 1% with sugar-free and acid-free flavoring are often helpful. Preventive dental measures include strict oral hygiene instruction and plaque control, topical fluoride applications, fluoride mouth rinses, and regular dental examinations, with restorative dental treatment when required. Oral candidiasis is a common complication of Sjögren's syndrome.

SYSTEMIC LUPUS ERYTHEMATOSUS

The oral mucosa and salivary glands may be involved in 15–50% of patients with SLE. The oral lesions include erythematous areas and ulcers on the lips or oral mucosa. Occasionally, oral ulcers may be the presenting feature, in which case biopsy and immunofluorescence microscopy may provide the diagnosis before systemic manifestations are discovered. A few patients with SLE have frank Sjögren's syndrome, although the incidence of subclinical involvement of salivary glands is probably much higher.

RHEUMATOID ARTHRITIS

In both adult and juvenile forms of rheumatoid arthritis, approximately 50% of cases involve the temporomandibular joint. Damage to the growth center in the developing mandibular condyle may lead to micrognathia and severe malocclusion. There is significant overlap between rheumatoid arthritis and Sjögren's syndrome.

PROGRESSIVE SYSTEMIC SCLEROSIS

Widening of the periodontal ligament spaces as shown on x-ray is found in about one-third of patients with systemic sclerosis. Furthermore, some patients also show resorption of some part of the mandibular angle or coronoid process. There may be limitation in opening the mouth, tongue rigidity, and dysphagia. This disease sometimes coexists with Sjögren's syndrome.

INFECTIOUS DISEASES

Many infectious diseases with immunologic features affect the mouth. Some, including tuberculosis, syphilis, and the acute viral diseases of childhood, have prominent oral features. Most of these diseases are adequately described elsewhere in this book. The 2 diseases described below—herpes simplex and candidiasis—are notable because the oral manifestations are often the most significant.

HERPES SIMPLEX VIRUS INFECTION

Major Immunologic Features

- Antibodies to herpes simplex virus appear during the primary infection and persist at lower levels throughout life.
- Cell-mediated immunity develops early during the primary infection.
- Cellular immune defects may be associated with recurrent infections.

General Considerations

Clinically detectable initial oral disease due to herpes simplex virus type 1 (HSV-1) takes the form of primary herpetic stomatitis. It is probable that many peo-

ple acquire the virus without experiencing the disease. Recurrent HSV-1 lesions are due not to exogenous reinfection but to reactivation of latent virus. They involve predominantly the lips (herpes labialis), but a rarer intraoral ulcerative form also occurs. The virus probably resides in neurons and in the cell bodies of the trigeminal ganglion or sensory root ganglia between attacks, although other sites for latent virus residence have not been excluded. Recurrence can be precipitated by sunlight, other viral infection, fever, stress, trauma, menstruation, and section of the sensory root of the trigeminal ganglion. There may be a connection between latent HSV-1 infection and oral squamous cell carcinoma (see below).

Immunologic Pathogenesis

Serum antibodies to HSV-1 reach maximum levels within 3 weeks of primary infection, with IgM preceding IgG. Cellular immune responses are first detectable as lymphocyte activation to herpes simplex virus antigen days before a significant antibody titer is found. Other indications of cell-mediated immunity follow weeks later. Persons prone to recurrent infections do not have higher or lower antibody levels than other individuals but may have a transient T cell-mediated immunodeficiency that encourages the virus. Macrophage activation probably contributes to control of the infection. Primary and secondary immunodeficiencies, including AIDS, are associated with severe forms of oral and other herpesvirus infections.

Clinical Features

Primary HSV-1 infections usually occur in children and young adults. When the disease affects older individuals, it is often opportunistic and should suggest immunodeficiency. The early phase is characterized by fever, dehydration, malaise, and nausea. The oral features are swelling and mobility of the gingival and oral mucosa, and crops of vesicles that rupture to form ulcers. There is excessive salivation, halitosis, dysphagia, and local lymphadenopathy. Individual lesions are 2–4 mm in diameter, painful, covered by a yellow pseudomembrane, and surrounded by a red margin. The acute primary phase usually lasts about 2 weeks, and the lesions subside without scar formation. Primary lesions in extraoral sites are occasionally seen as a result of inoculation in dentists and other clinicians and are due to virus in the saliva of patients.

Recurrent Herpes Simplex. This condition is much more common than primary herpetic stomatitis, and the lesions are usually confined to the external surface of the lips (cold sore), although an intraoral form affecting the mucosa of the hard palate or gingivae is also seen. The lesion starts as a vesicle or group of vesicles after a short period of tingling or burning sensation. The vesicle usually crusts over and heals within 10 days. Precipitating factors include local trauma, emotional stress, menstruation, exposure to sunlight, fever, other viral infections, and immunodeficiency, including AIDS.

Immunologic Diagnosis

Diagnosis of the primary disease is usually made on clinical grounds and may be confirmed by cytologic examination of the fluid from an intact vesicle. Viral intranuclear inclusion bodies and giant cells may be seen. Viral culture may be performed, but a positive result is not necessarily diagnostic of the disease because HSV-1 can also be found in healthy controls. A rise in antibody titer and the inception of lymphocyte responses to viral antigen during the disease are occasionally helpful in the diagnosis.

Immunologic methods are not particularly helpful in the diagnosis of recurrent herpes. Antibody is always found in sufferers from recurrent herpes and does not appear to be protective, since titers do not change during recurrent episodes. There appears to be a correlation between episodes of recurrent herpes labialis and gamma interferon production.

Treatment & Prognosis

Treatment is usually directed toward symptomatic relief, with tetracycline mouth rinses occasionally being used to reduce secondary infection. Antiviral agents that have been tried include idoxuridine, cytarabine, vidarabine (adenine arabinoside [Ara-A]), acyclovir, and interferon. There are as yet insufficient data to justify the routine use of these drugs for oral herpes simplex. Several specific antiherpes drugs are now undergoing clinical trials. The use of dyes combined with fluorescent light is contraindicated because of the danger of potentiating the oncogenic properties of HSV-1.

ORAL CANDIDIASIS

Major Immunologic Features

- Oral lesions due to *Candida albicans*.
- Many associated immunologic defects.
- The most significant oral indication of an underlying immunodeficiency. A prominent feature of AIDS virus-induced immunodeficiency.

General Considerations

Oral candidiasis is the most common oral fungal disease. It may occur in acute or chronic form at any age. The disease may be a sign of serious life-threatening systemic disease or may be confined to a small part of the oral mucous membrane and be of no general significance. *Candida* species are frequent oral commensals, and it has not yet been established whether candidiasis is predominantly of endogenous or exogenous origin.

Classification

A valuable classification of oral candidiasis has been provided by Lehner:

A. Acute Pseudomembranous Candidiasis (Thrush): This disease, predominantly found in infants and in adults with malignant disease and diabetes, is characterized by the presence of masses of

loose, white, curdlike collections of fungus and exudate on the oral mucosa. These can be detached to leave a red, bleeding surface. The condition can also be caused by immunosuppressive drugs and systemic antibiotics. A variant is frequently seen in individuals with AIDS or ARC (see Chapter 20).

B. Acute Atrophic Candidiasis: A rarer complication of broad-spectrum antibiotic therapy, this disease presents as a soft, smooth glossitis and angular cheilitis, sometimes involving other areas of the oral mucous membrane.

C. Chronic Atrophic Candidiasis (Denture Stomatitis): This disease is sufficiently common to warrant separate discussion (see below).

D. Chronic Mucocutaneous Candidiasis: Four clinical subtypes are described.

1. Chronic oral hyperplastic candidiasis (candidal leukoplakia)–Lesion limited to the mouth, taking the form of a firm, white area of mucosa that is clinically difficult to distinguish from other forms of leukoplakia.

2. Chronic localized mucocutaneous candidiasis–Long-lasting oral candidiasis, usually starting in childhood and spreading to involve the nails and other areas of skin.

3. Chronic localized mucocutaneous candidiasis with granuloma–Similar to (2) above, starting in infancy and characterized by candidal granuloma of face and scalp.

4. Chronic localized mucocutaneous candidiasis with endocrinopathy–Mucocutaneous candidiasis with, for example, hypoparathyroidism, Addison's disease, pernicious anemia or hypothyroidism, or a combination of endocrine disorders.

Immunologic Pathogenesis

The immunologic features of generalized candidiasis are discussed elsewhere (see Chapters 20, 29, and 30). A wide range of immunologic defects have been found, including defects in cytotoxicity to *Candida*, reduced lymphokine production, failure of anticandidal antibody response of one or more classes, generalized cytotoxicity defects, failure of lymphocyte activation to candidal antigen, absence of delayed hypersensitivity skin test to *Candida* or to many antigens, and presence of an abnormal suppressor cell population. However, immunologic defects alone do not explain the pathogenesis of candidiasis. High glucose levels in diabetics and low levels of serum iron transferrin and blood folate are also important factors. Granulocyte defects have been shown in some patients, as have defects in leukocyte myeloperoxidase. A few patients have been described in whom antibody production to *Candida* and other antigens was raised while cellular immune function was depressed.

Clinical Features of Some Oral Candidal Lesions

A. Pseudomembranous Candidiasis (Thrush): Thrush can be seen in infants, in debilitated patients, in AIDS and ARC, and in patients treated with antibiotics, corticosteroids, immunosuppressive drugs, and radiation therapy to the head and neck region. Creamy whitish-yellow patches cover variable areas of the oral mucous membrane and can be rubbed or lifted off, leaving an erythematous bleeding base. The patient may not notice the lesion or may complain of soreness or dryness. There may be associated pharyngeal or esophageal candidiasis.

B. Acute Atrophic Candidiasis: This lesion is painful and presents as smooth, depapillated tongue with localized erosions. It may follow thrush or may occur de novo. It is particularly associated with antibiotic therapy.

C. Chronic Hyperplastic Candidiasis (Candidal Leukoplakia): Persistent white areas are found on the buccal mucous membrane and other areas of the oral mucosa. These may be soft or firm and cannot be readily detached. They may closely resemble other forms of leukoplakia, but it is important that they be diagnosed because of the potential for successful treatment with antifungal drugs. Since the question of malignant transformation in candidal leukoplakia has not been settled, for the moment it is best to assume long-term malignant potential for the untreated lesion.

The clinical features of mucocutaneous candidiasis, candidal granuloma, and mucocutaneous candidiasis with endocrinopathy are discussed elsewhere (see Chapters 20, 29, and 30). All forms of oral candidiasis are seen in AIDS.

Immunologic Diagnosis

The diagnosis of thrush is based upon the clinical appearance and history. The immunologic approach is directed toward establishing the nature of the immunologic defect, if any. The same remarks apply to acute atrophic candidiasis. Although immunologic investigation of patients with oral candidiasis is still predominantly a research tool, it is likely that subtypes will be identified and that specific immunologic treatment will be directed toward the correction of localized defects in cell-mediated immunity. The differential diagnosis of candidal leukoplakia from other white oral lesions involves smear, culture, and biopsy.

Treatment & Prognosis

Treatment of localized oral candidiasis consists of elimination of predisposing factors, where known, and administration of topical antifungal therapy. This may be prolonged in the case of chronic forms of oral candidiasis. Systemic therapy is used in cases that are resistant to local measures and in generalized mucocutaneous candidiasis. The treatment of denture stomatitis is discussed below.

DENTURE STOMATITIS

Major Immunologic Features

- Inflammation of mucous membrane under dentures.

- Associated with *Candida albicans*.
- Some evidence of impaired cell-mediated responses to *Candida*.
- Restoration of impaired cell-mediated responses with antifungal treatment.

General Considerations

Inflammation of the mucous membrane under dentures is a common problem. The condition can occur with any form of intraoral prosthesis, but removable maxillary full dentures produce the largest number of cases. The mucous membrane is bright red, soft, and somewhat spongy. It is not always possible to isolate *Candida* from the mucous membrane by smear, scraping, or biopsy, but the fungus can usually be cultured from the plaquelike material covering the fitting surface of the denture. It is not clear whether the inflammatory reaction is due to tissue invasion by a small number of organisms, the effects of toxins released by the fungus, or a hypersensitivity reaction to *Candida* antigens.

Immunologic Pathogenesis

Dentures cause a continual mild trauma to the mucous membrane that may facilitate the entry of candidal antigens into the tissues. This effect may be aggravated by the denture-induced obstruction to salivary flow across the mucosa and by reduced elimination of affected epithelial cells. Another factor could be competition by microbial species under the denture for the limited nutrients available in this location. Thus, the condition can be exacerbated by long-term treatment with antibiotics. Salivary IgA antibodies to *C albicans* may be involved in the normal defense mechanism to this organism, and dentures could prevent access of this immunoglobulin to its target. Serum anticandidal antibodies are associated with denture stomatitis but are apparently not protective. On the other hand, cell-mediated immune mechanisms seem to be important in the normal response to *Candida*. There is some evidence that the incidence of positive skin reaction to *Candida* extract is lower in denture stomatitis patients than in controls. Furthermore, defective cellular hypersensitivity to *C albicans* can be restored after successful antifungal treatment. This suggests that *Candida*-induced denture stomatitis might be the cause of the suppression of the cellular immune response rather than the converse. However, the apparent decrease in cell-mediated immunity to candidal antigen could be due to the withdrawal of sensitized lymphocytes from the circulation into the lesion.

Clinical Features

Denture stomatitis consists of inflammation on the denture-bearing surface of the oral mucous membrane and appears more frequently in the maxillary than in the mandibular area. The severity ranges from a localized area of tiny red dots through diffuse erythema to a proliferative response which may result in papillary hyperplasia. The condition may be associated with angular cheilitis and glossitis. There may be no symptoms, or the patient may complain of a burning sensation under the denture.

Immunologic Diagnosis

The diagnosis is usually afforded by the location of the lesion and the clinical appearance; immunologic techniques are not used on a routine basis. However, as with other forms of oral candidiasis, it is always wise to bear in mind the possibility of an underlying immunodeficiency, particularly when the lesion arises in an otherwise previously healthy mouth.

Treatment

Local treatment is usually effective and consists of sterilization of the denture in antiseptic solution and administration of nystatin troches (500,000 units 3 times daily). It is usually necessary to ask the patient not to wear the denture during the period of antifungal therapy, and it may be necessary to replace the ill-fitting denture with a more satisfactory prosthesis. The condition is frequently recurrent and may be intractable. Systemic spread of denture candidiasis has not been documented.

ORAL TUMOR IMMUNOLOGY

Cancer of the mouth is one of the more common forms of malignant disease. Approximately 24,000 new cases are reported each year in the USA. About 3% of cancer deaths in men and 1% of cancer deaths in women have been attributed to cancer of the mouth, pharynx, and larynx.

The most important factors contributing to oral squamous cell carcinoma appear to be tobacco habits, particularly smoking. Alcohol consumption may play a part, though this is less clearly defined. Recently, attention has turned to 2 other groups of factors that may contribute to the cause and pathogenetic development of oral cancer: cell-mediated immune deficiency and virus infection, both herpes simplex virus and papillomavirus.

Impaired cellular immunity, as indicated by DNCB sensitization and mitogen activation of lymphocytes, has been found in patients with squamous cell carcinoma of the head and neck as well as other sites. Decreased percentages and numbers of circulating T cells have also been shown in these patients, as have increases in T cell numbers in comparison with pretreatment values in patients who respond to radiotherapy. Furthermore, there is evidence that T cell numbers fall at the development of metastasis. The reduced cell-mediated immune responses may be due, at least in part, to the presence of suppressor cells, probably macrophages.

Preliminary reports suggest that cell-mediated immune responses to HSV-1 are depressed in patients

with oral leukoplakia who show only benign hyperkeratosis, whereas in patients in whom the histologic changes suggest a premalignant state (epithelial atypia) the lymphocyte activation response to HSV-1 is raised to levels comparable to those seen in active primary or recurrent herpetic infection. Evidence has been advanced to suggest that a fall in lymphocyte response to HSV-1 may precede the development of carcinoma in situ in a preexisting benign hyperkeratosis. However, the series of patients studied was very small, and these results await confirmation. Other studies indicate that smokers have higher levels of antibody to HSV-1 than nonsmokers, and this may be important since oral cancer is more common in smokers. A possible pathogenesis for oral cancer is that cigarette smoke reactivates latent HSV-1 and then assists integration of transforming genes into epithelial cells. Patients with oral cancer have IgM antibody to HSV-1, possibly directed against a T-independent antigen. Efforts are being made to identify such an antigen, although with only partial success so far. Recently, RNA complementary to HSV has been found in oral cancer tissue. Several reports have suggested the presence of papillomavirus DNA in dysplastic oral epithelium and even in oral cancer tissue.

In view of the rapidly accumulating evidence for oncogenic potential of herpes and other viruses and the relationship between host responses and the development and progress of cancer, this field of study can be expected to grow and to yield new methods of assessing prognosis and progress of head and neck cancer.

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Immunization

37

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IMMUNIZATION AGAINST INFECTIOUS DISEASES

It has been recognized for centuries that individuals who recover from certain diseases are protected from recurrences. The moderately successful but hazardous introduction of small quantities of fluid from the pustules of smallpox into the skin of uninfected persons (variolation) was an effort to imitate this natural phenomenon. Jenner's introduction of vaccination with cowpox (1796) to protect against smallpox was the first documented use of a live attenuated viral vaccine and the beginning of modern immunization. Koch demonstrated the specific bacterial cause of anthrax in 1876, and the etiologic agents of several common illnesses were rapidly identified thereafter. Attempts to develop immunizing agents followed (Table 37–1).

Types of Immune Response

Immunization results in the production of antibodies directed against the infecting agent or its toxic products; it may also initiate cellular responses mediated by lymphocytes and macrophages. The most important protective antibodies include those which inactivate soluble toxic protein products of bacteria (antitoxins), fascilitate phagocytosis and intracellular digestion of bacteria (opsonins), interact with the components of serum complement to damage the bacterial membrane with resultant bacteriolysis (lysins), or prevent proliferation of infectious virus (neutralizing antibodies). Newly appreciated are those antibodies that interact with components of the bacterial surface to prevent adhesion to mucosal surfaces (antiadhesins). Some antibodies may not be protective and, by "blocking" the reaction of protective antibodies with the pathogen, may actually depress the body's defenses.

Antigens react with antibody in the bloodstream and extracellular fluid and at mucosal surfaces. Antibodies cannot readily reach intracellular sites of infection as are found with viral replication. However, they are effective against many viral diseases (1) by interacting with virus before initial intracellular penetration occurs, and (2) by preventing locally replicating virus from disseminating from the site of entry to an important target organ, as in the spread of poliovirus from the gut to the central nervous system or of rabies from a puncture wound to peripheral neural tissue. Lymphocytes acting alone and antibody interacting with lymphoid or monocytic effector K cells may also recognize surface changes in virus-infected cells and destroy these infected "foreign" cells.

Table 37–1. Historical milestones in immunization.

Variolation	1721
Vaccination	1796
Rabies vaccine	1885
Diphtheria toxoid	1925
Tetanus toxoid	1925
Pertussis vaccine	1925
Viral culture in chick embryo	1931
Yellow fever vaccine	1937
Influenza vaccine	1943
Viral tissue culture	1949
Poliovaccine, inactivated (Salk)	1954
Poliovaccine, live, attenuated (Sabin)	1956
Measles vaccine	1960
Tetanus immune globulin (human)	1962
Rubella vaccine	1966
Mumps vaccine	1967
Hepatitis B vaccine	1975
Licensure of first recombinant vaccine (hepatitis B)	1986

Passive Immunization

Immunization may be accomplished passively by administering either preformed immunoreactive serum or cells, or actively by presenting a suitable antigenic stimulus to the host's own immune system.

Antibody—either as whole serum or as fractionated, concentrated immune (gamma) globulin that is predominantly IgG—may be obtained from donors who have recovered from an infectious disease or have been immunized. These antibodies may provide immediate protection to an antibody-free individual. Passive immunization is thus useful for individuals who cannot form antibodies or for the normal host who might develop disease before active immunization could stimulate antibody production, which usually requires at least 7–10 days.

Antibody may be obtained from humans or animals, but animal sera are always less desirable, since nonhuman proteins themselves give rise to an immune response that leads to rapid clearance of the protective molecules from the circulation of the recipient and may even result in clinical illness (serum sickness; see Hazards of Passive Immunization, below). Thus, to obtain a similar protective effect, a much greater quantity of animal antiserum must be injected compared to

human antiserum, eg, 3000 units of equine tetanus antitoxin versus 300 units of human tetanus immune globulin.

No antiserum of animal origin should be given without carefully inquiring about prior exposure or allergic response to any product of the specific animal source. Patients with an unrelated allergy are probably more prone to develop serum reactions. Whenever a foreign antiserum is administered, a syringe containing aqueous epinephrine 1:1000 should be available, and eye or scratch testing (see Chapter 24) should be followed by intracutaneous testing for hypersensitivity. If allergy is present by history or test and no alternative to serum therapy is possible, a patient may sometimes be given an essential medication to tolerance in repeated fractional doses of progressively increasing size. Simultaneous administration of antihistamines, corticosteroids, and even epinephrine may be necessary during this procedure of "desensitization," which may simply limit the allergic reactions to an acceptably small magnitude.

Persistence of certain human antibodies, eg, to varicella-zoster, is short-lived, and zoster immune globulin (ZIG) must therefore be prepared from the sera of convalescent zoster patients. By contrast, antibody to measles and hepatitis A is so ubiquitous in the population at large that normal immune globulin (IG) will usually prevent or modify clinical illness with these infections if given early in the incubation period (Table 37–2). Table 37–3 lists antisera generally available for passive immunization at present.

In the preantibiotic era, passive immunization was administered with some success as therapy for pneumococcal or *Haemophilus* infection. The need to identify the infecting serotype and obtain the appropriate type-specific antiserum, the illness caused by injection of the foreign proteins, and the relatively poor therapeutic response led to the prompt abandonment of this unsatisfactory method of treatment as soon as effective antimicrobial chemotherapy became available. However, the availability of vaccine-induced human hyperimmune globulin and the continued high mortality rate associated with infections with antibiotic-sensitive pneumococci, group B streptococci, and *H influenzae* have reopened the question of serum therapy. For the present, serum therapy for established illness is largely limited to the administration of antivenins and of botulinus, tetanus, and diphtheria antitoxins to block attachment of yet unbound toxin.

In the absence of demonstrably low serum IgG or (rarely) specific antibody deficiencies, the administration of IG is of no value in the prevention of recurrent infections.

Table 37–2. Use of HBIG and HBV following percutaneous or mucosal exposure to possibly HBsAG-positive material.*

Source	Exposed Person	
	Unvaccinated	Vaccinated
Known HBsAg-positive	HBIG once immediately. Begin HBV series.	Test exposed person for anti-HBs;† if negative, give HBIG once immediately and HBV booster.
Known high risk of being HBsAg-positive	Begin HBV series. Test source for HBsAg, and give HBIG once if positive.	Test source only if exposed person fails to respond to vaccine; if source is HBsAg-positive, give HBIG once plus HBV booster.
Known low risk of being HBsAg-positive *or* Unknown	Begin HBV series.	No action required.

*Adapted from recommendations of the CDC Advisory Committee on Immunization Practices.

†Test results obtained within the past year may be used.

Passive Transfer of Cellular Immunity

Antibodies produced following some infections, particularly those due to mycobacteria, fungi, and many viruses, fail to protect against infection. Rather, interaction of immune lymphocytes and macrophages largely determines recovery from these illnesses. Attempts have been made to transmit this cell-mediated immunity, eg, to vaccinia virus in the progressively infected, immunologically incompetent host; to *Coccidioides immitis* in the patient with disseminated coccidioidomycosis; and to *Mycobacterium leprae* in lepromatous leprosy (see Chapter 30). Whole blood, leukocyte-rich buffy coat, and leukocyte-derived transfer factor have been utilized. The value of this type of therapy is uncertain, and these procedures are still experimental.

Hazards of Passive Immunization

Illness may arise from a single injection of foreign serum but more commonly occurs in patients who have previously been injected with proteins from the same or a related species. Reactions range in severity from acute anaphylaxis with hives, back pain, dyspnea, cardiovascular collapse, and even death to serum sickness arising hours to weeks following treatment. Typical manifestations of serum sickness include adenopathy, urticaria, arthritis, and fever. Demyelinating encephalopathy has been reported. Rarely, the administration of human IG is attended by similar allergic reactions, particularly in patients who are congenitally deficient in one or more immunoglobulins but still capable of mounting an immune response. Hepatitis A, B, or "C" (non-A, non-B) may be transmitted by whole human plasma or serum, but purified IG is free of hepatitis.

Note: Great care must be exercised in administering standard IG to avoid accidental intravenous injection. Currently, most human and animal IG preparations are given by the intramuscular route. They all contain high-molecular-weight aggregated IgG, intravenous administration of which will frequently result in moderate to severe anaphylactic reactions with possible vasomotor collapse and death. Standard IG must

Table 37–3. Materials available for passive immunization. (All are of human origin unless otherwise stated.)

Disease	Product	Dosage	Comments
Black widow spider bite	Antivenin widow spider, equine.	1 vial IM or IV.	A second dose may be given if symptoms do not subside in 3 hours.
Botulism	ABE polyvalent antitoxin, equine.	1 vial IV and 1 vial IM; repeat after 2–4 hours if symptoms worsen, and after 12–24 hours.	Available from CDC.* 20% incidence of serum reactions. Only type E antitoxin has been shown to affect outcome of illness. Prophylaxis is not routinely recommended but may be given to asymptomatic exposed persons.
Diphtheria	Diphtheria antitoxin, equine.	20,000–120,000 units IM depending on severity and duration of illness.	Active immunization and perhaps erythromycin prophylaxis should be given to nonimmune contacts of active cases rather than antitoxin prophylaxis. Contacts should be observed for signs of illness so that antitoxin may be administered if needed.
Hepatitis A	Immune globulin.	0.02 mL/kg IM as soon as possible after exposure up to 2 weeks. A protective effect lasts about 2 months.	Modifies but does not prevent infection. Recommended for sexual and household contacts of infected persons, including diapered children and their staff contacts in a child-care center if one case occurs among them or if cases are recognized in the households of more than 2 children. (If cases occur in more than 3 homes, consider prophylaxis for all households with diapered children attending the center.) In centers without diapered children, prophylaxis is recommended only for classroom contacts of an index case. Prophylaxis is also suggested for coworkers of an infected food handler (but not generally for patrons) and for persons exposed to a common source *if* cases have not yet begun to occur. Prophylaxis is not recommended for personal contacts at offices, schools, hospitals, or institutions for custodial care *except* in an outbreak centered in these areas.
		For continuous risk of exposure, a dose of 0.06 mL/kg is recommended every 5 months.	Personnel of mental institutions, facilities for retarded children, and prisons appear to be at chronic risk of acquiring hepatitis A, as are those who work with nonhuman primates. Also recommended for travelers who will remain in endemic areas for over 2 months.
Hepatitis B	Hepatitis B immune globulin (HBIG).	0.06 mL/kg IM up to a maximum of 5 mL as soon as possible after exposure, preferably within 24 hours, but up to 14 days for sexual exposure. HBV vaccination begun within 7 days of exposure is recommended in preference to a second HBIG injection 25–30 days after the first.	Administer to nonimmune individuals as postexposure prophylaxis following sexual contact with HBsAg-positive individuals (one dose of HBIG appears to be as effective as 2 doses for sexual exposure). For percutaneous or mucosal exposure to known HBsAg-positive or high-risk material, prophylactic strategy depends upon testing the source and upon the vaccination history of the exposed person (see Table 37–4). Of no value for persons already demonstrating anti-HBsAg antibody. Administration of various live virus vaccines should be delayed for at least a couple of months after this concentrated immune globulin has been given. Pregnant women at high risk for carriage of HBsAg should be screened before delivery or as soon as possible thereafter, and newborn infants of all carriers should be given HBIG, 0.5 mL, within 12 hours of birth. Immunization with HBV vaccine should be started within the first week of life (see Table 37–4). High-risk mothers include women of Asian, Pacific island, or Alaskan Eskimo descent, whether immigrant or US-born; women born in Haiti or sub-Saharan Africa; and women with a history of acute or chronic liver disease, work or treatment in a hemodialysis unit, work or residence in an institution for the mentally retarded, repeated blood transfusions, frequent occupational exposure to blood in a medicodental setting, rejection as a blood donor, household exposure to an HBV carrier or a hemodialysis patient, multiple episodes of sexually transmitted disease, or percutaneous use of illicit drugs. HBIG has no effect upon non-A, non-B hepatitis.
Hypogammaglobulinemia.	Immune globulin.	0.6 mL/kg IM every 3–4 weeks.	Give double dose at onset of therapy. Immune globulin is of no value in the prevention of frequent respiratory infections in the absence of demonstrable hypogammaglobulinemia.
	Immune globulin IV	100–150 mg/kg IV about once a month, depending on maintenance of serum IgG levels.	Ordinary immune globulin cannot safely be given intravenously because of complement-activating aggregates. It is difficult to administer sufficient intramuscular globulin to maintain normal IgG levels in immunodeficient children or to passively protect acutely infected individuals who lack a specific antibody. This material contains a very small amount of IgA and can cause an allergic reaction in a sensitive IgA-deficient recipient. The IgE in some preparations may cause some reactions; although one product causes symptoms, particularly if confirmed by skin testing, another preparation may not.

*See footnotes on p 673.

Table 37–3 (cont'd). Materials available for passive immunization.

Disease	Product	Dosage	Comments
Measles	Immune globulin.	0.25 mL/kg IM as soon as possible after exposure. This dose may be ineffective in immunoincompetent patients, who should receive 20–30 mL.	Live measles vaccine will usually prevent natural infection if given within 48 hours following exposure. If immune globulin is administered, delay immunization with live virus for 3 months. Do not vaccinate infants under age 15 months.
Rabies	Rabies immune globulin. (Antirabies serum, equine, may be available but is much less desirable.)	20 IU/kg, 50% of which is infiltrated locally at the wound site if anatomically feasible, and the remainder given IM. (See also rabies vaccine in Table 37–4.) If the equine product is used, the dose is 40 IU/kg.	Give as soon as possible after exposure. Recommended for all bite or scratch exposures to carnivores, especially bat, skunk, fox, coyote, or raccoon, despite animal's apparent health, if the brain cannot be immediately examined and found rabies-free. Give also even for abrasion exposure to known or suspected rabid animals as well as for bite (skin penetration by teeth) of escaped dogs and cats whose health cannot be determined. Not recommended for individuals with demonstrated antibody response from preexposure prophylaxis.
Rh isoimmunization (erythroblastosis fetalis)	Rh_0 (D) immune globulin.	1 dose IM with 72 hours of abortion, amniocentesis or chorionic villus biopsy, obstetric delivery of an Rh-positive infant, or transfusion of Rh-positive blood in an Rh_0 (D)-negative female.	For nonimmune females only. May be effective at much greater postexposure interval. Give even if more than 72 hours have elapsed. One vial contains 300 μg antibody and can reliably inhibit the immune response to a fetomaternal bleed of 7.5–8 mL as estimated by the Betke-Kleihauer smear technique. Some groups also recommend administration of 100 μg of antibody at 28 and 34 weeks of pregnancy to prevent prepartum isoimmunization. Transient seropositivity for anti-HAV and anti-HBV antibodies may follow the administration of large doses.
Snakebite	Antivenin coral snake, equine. Antivenin rattlesnake, copperhead, and moccasin, equine.	At least 3–5 vials IV.	Dose should be sufficient to reverse symptoms of envenomation. Consider antitetanus measures as well.
Tetanus	Tetanus immune globulin. (Bovine and equine antitoxins may be available but are not recommended. They are used at 10 times the dose of tetanus immune globulin.)	Prophylaxis: 250–500 units IM. Therapy: 3000–6000 units IM.	Give in separate syringe at separate site from simultaneously administered toxoid. Recommended only for major contaminated wounds in individuals who have had fewer than 2 doses of toxoid at any time in the past (fewer than 3 doses if wound is more than 24 hours old). (See tetanus toxoid in Table 37–4.) There is some evidence that 250 units given intrathecally to mildly affected patients prevents progression to severe disease and death, but intrathecal therapy is not effective in severe cases with generalized repeated spasms.
Vaccinia	Vaccinia immune globulin (VIG). (Available from CDC.*)	Prophylaxis: 0.3 mL/kg IM. Therapy: 0.6 mL/kg IM. VIG may be repeated as necessary for treatment and at intervals of 1 week for prophylaxis.	Give at a different site if used to prevent dissemination in a patient with skin disease who must undergo vaccination. May be useful in treatment of vaccinia of the eye, eczema vaccinatum, generalized vaccinia, and vaccinia necrosum and in the prevention of such complications in exposed patients with skin disorders such as eczema or impetigo. Also recommended to prevent fetal vaccinia when a pregnant woman must be vaccinated. VIG should rarely be needed, since smallpox vaccination is now limited to military personnel and at-risk laboratory workers.

Table 37–3 (cont'd). Materials available for passive immunization.

Disease	Product	Dosage	Comments
Varicella	Varicella-zoster immune globulin (VZIG).	1 vial/10 kg or fraction thereof, up to a maximum of 5 vials, given IM within 96 hours of exposure.	†Should be administered to nonimmune leukemic, lymphomatous, or immunosuppressed children, children receiving prednisone at ≥ 2 mg/kg/d for any reason, or other immunoincompetent children < 15 years of age who have had household, hospital (same room containing ≤ 4 beds or adjacent beds in large ward), or playmate (> 1 hour play indoors) contact with a known case of varicella-zoster. Should also be given to exposed bone marrow transplant patients regardless of immune history of donor, to exposed infants born before 28 weeks of gestation, and to neonates whose mothers have developed varicella < 5 days before or 48 hours after delivery or who are exposed postnatally and whose mothers have uncertain or negative histories of varicella. VZIG should be considered for adults—especially pregnant women and immunocompromised patients—having close contact with a case of varicella-zoster as defined above for children and whose history suggests susceptibility. A negative history for varicella is extremely unreliable (only about 8% of adults who believe they are nonimmune become infected after household exposure) and should if possible be checked by a serologic test such as the fluorescent antibody test against membrane antigen, because of the very high cost of VZIG (nearly $400 for an adult dose in August, 1983). Protective antibody levels can be attained using immune globulin IV in a dose of 6 mL/kg if VZIG is unavailable.

*Centers for Disease Control—Telephone: (404) 329–3311 (main switchboard, day) or (404) 329–3644 (night).
†Contact the regional blood center of the American Red Cross.
Note: Passive immunotherapy or immunoprophylaxis should always be administered as soon as possible after exposure to the offending agent. Immune antisera and globulin are always given intramuscularly unless otherwise noted. Always question carefully and test for hypersensitivity before administering animal sera.

not be confused with a recently licensed product, immune globulin IV, which *can* be given safely intravenously.

The administration of intact lymphocytes to promote cell-mediated immunity is also hazardous if the recipient is too immunologically depressed to prevent implantation of incompatible donor cells. The engrafted donor cells may "reject" the recipient by GVH reaction, producing rash, pancytopenia, fever, diarrhea, hepatosplenomegaly, and death (see Chapter 22).

Active Immunization

Primary active immunity develops more slowly than the incubation period of most infections and must therefore be induced prior to exposure to the etiologic agent. One exception (no longer of practical importance) is vaccinia-induced immunity to smallpox, which takes only 10 days as opposed to the 14-day incubation period of the virulent infection. By contrast, "booster" reimmunization in a previously immune individual provides a rapid secondary (anamnestic) increase in serum antibody that outpaces the development—to give one example—of tetanus from a contaminated wound.

Active immunization may be achieved with either living or dead materials. Nonviable antigens usually are either structural components of the infecting organism which induce antibodies that prevent infection, or detoxified bacterial products (toxoids) which stimulate antitoxins that prevent illness without directly inhibiting the pathogen. Although tetanus toxoid provides a particularly long-lasting immunity of at least 10 years' duration, most nonliving vaccines provide protection for only a limited time. Repeated injections are needed to maintain even a moderate level of protection against influenza, plague, cholera, and typhoid fever. Not even natural infection always results in durable immunity. Examples include repeated, although perhaps milder, attacks of illness with *Mycoplasma pneumoniae* and respiratory syncytial virus as well as cholera.

Previous infection can also substantially alter the response to an inactivated vaccine. For example, volunteers who have recovered from cholera or who live in a cholera-endemic area respond to parenteral immunization with an increase in anti-cholera secretory IgA. This increase is not seen in immunized control subjects.

The route of immunization may be an important determinant of successful vaccination, particularly if nonreplicating immunogens are employed. Thus, immunization intranasally or by aerosol often appears more successful than parenteral injection against viral or bacterial challenges to the respiratory tree.

Splenectomy may markedly impair the response to thymus-independent antigens such as bacterial polysaccharides, although many splenectomized patients respond normally to polysaccharide antigens because of priming by natural exposure prior to splenectomy. These patients respond poorly, however, to polysaccharide antigens encountered after surgery.

Active immunization with living agents is generally preferable to immunization with killed vaccines because of a superior and long-lived immune response. A single dose of a live, attenuated virus vaccine often suffices for reliable immunization. Multiple immunizations are recommended for polio in case intercurrent enteroviral infection or interference among 3 simultaneously administered virus types in the trivalent vaccine prevents completely successful primary immunization. The durability of immunity to many viral infections is unexplained and may include repeated natural reexposure to new cases in the community, the unusually large antigenic stimulus that infection with a living agent provides, or other mechanisms such as the persistence of latent virus.

All immunizing materials—but live agents particularly—must be properly stored to retain effectiveness. Serious failures of smallpox and measles immunization have resulted from inadequate refrigeration prior to use. Agents presently licensed for active immunization are listed in Table 37–4.

Hazards of Active Immunization

The physician should defer immunization of a severely febrile patient, partly to avoid additional effects of the vaccine but mainly to ensure that a worsening of the patient's condition is not erroneously attributed to the vaccine. Active immunization may cause fever, malaise, and soreness at injection sites. Some reactions are relatively specific for the immunizing agent, such as arthralgia/arthritis following rubella vaccine or convulsions following pertussis vaccine, but are much less frequent and less severe than those accompanying unmodified natural illness. Reactions known to be associated with a particular product are described in the manufacturer's package insert, the *Physicians' Desk Reference*, and standard texts.

Repeated immunization, particularly with diphtheria and tetanus toxoids, may result in increasingly severe local reactions. Diphtheria antigen in adult-type combined diphtheria-tetanus toxoid (Td) is therefore 5- to 10-fold less than in childhood DTP, and a lower frequency of booster immunization for tetanus is now recommended than in the past. Although experimentally hyperimmunized animals display a variety of adverse effects, including amyloidosis and cancer, and immunization has been suspected of precipitating systemic lupus erythematosus in humans, follow-up of intensively immunized individuals over a 15-year period has not shown any clinical sequelae. Antibodies to certain bacteria reportedly cross-react with mammalian tissues, suggesting the potential hazard of autoimmune disease following extensive immunization, but the clinical significance of such experimental observations is unknown.

The careful monitoring system established during the 1976 "swine flu" immunization program revealed a 5-fold increase in postimmunization Guillain-Barre syndrome in comparison with unvaccinated controls. This complication arises within 10 weeks of immunization and has resulted in a 5% mortality rate, with another 5–10% of patients displaying residual weakness. The rarity of this complication (one case per 85,000) would not permit recognition in field trials or in the absence of a surveillance program and demonstrates the difficulty in accurately assessing the risks of immunization. The unexpected complications of the "swine flu" immunization program and mounting concern about the possible impact of lawsuits for vaccine-related damage upon the present and future availability of old and new vaccines have led to the establishment of an ongoing nationwide Monitoring System for Adverse Events Following Immunization. Episodes determined by the local health department to (1) have occurred within 30 days of receipt of a publicly funded vaccine (50% of total usage) and (2) be sufficiently serious to result in a visit to a physician or hospitalization are reported in detail to the state health departments and hence to the Centers for Disease Control. The latter determines crude reaction rates and investigates unusual types or clusters of reactions. Reports from all sources are encouraged. Over a period of 4 years, 78 deaths were reported, 63 of which could readily be explained without relation to vaccination.

Allergic reactions may occur on exposure to egg protein (measles, mumps, influenza, yellow fever) or antibiotics or preservatives, eg, neomycin or mercurials, in viral vaccines. With the exception of the relatively impure yellow fever vaccine grown in eggs, the quantity of nonviral antigen in a vaccine cell culture is usually insufficient to elicit a response in the allergic individual, but a patient known to exhibit an intense response such as anaphylaxis to a vaccine component should not receive the vaccine. (Occasionally, the product of a different manufacturer does not contain the offending allergen.) Successful desensitization has, however, been reported. Improvements in antigenicity and better purification procedures in vaccine production decrease the amount and number of foreign substances injected and result in fewer side effects.

Unique Hazards of Live Vaccines

Because of their potential effect upon the fetus, live vaccines should *not* be given to a pregnant woman unless there is a high immediate risk (eg, a poliomyelitis epidemic). A pregnant woman traveling to an area endemic for yellow fever *should* be immunized because the risk of infection exceeds the small theoretical hazard to fetus and mother. If yellow fever vaccination is being performed solely to comply with a legal requirement for international travel, however, the woman should seek a waiver with a letter from her physician. Live vaccines, furthermore, can cause serious or even fatal illness in an immunologically incompetent host. They should not be given to patients receiving corticosteroids, alkylating drugs, radiation, or other immunosuppressive agents, nor should they be given to individuals with known or suspected congenital or acquired defects in cell-mediated immunity, as seen in severe combined immunodeficiency disease or leukemias or lymphomas, including Hodgkin's disease. Patients with pure hypogammaglobulinemia but

Table 37–4. Materials available for active immunization.*

Disease	Product (Source)	Type of Agent	Route of Administration	Primary Immunization	Duration of Effect	Comments
Cholera	Cholera vaccine.	Killed bacteria	Subcut, IM, intradermal	2 doses 1 week or more apart.	6 months†	50% protective; International Certificate may be required for travel.
Diphtheria	DTP, DT (adsorbed) for child under age 7; Td (adsorbed) for all others.	Toxoid	IM	3 doses 4 weeks or more apart, with an additional dose 1 year later for a child under age 7. (Can be given at same time as polio vaccine if doses at least 8 weeks apart.) A fifth dose before entering school is recommended if the fourth dose was given before the fourth birthday.	10 years‡	Do not use DTP after the seventh birthday or if convulsions follow its use (see comments below under Pertussis). Recipients aged 7 and above should be given a third dose 6–12 months after the second. The need for boosters after age 18 is uncertain. Children previously immunized to DTP and now undergoing chemotherapy for neoplastic disease generally respond to a booster.
Haemophilus influenzae influenza	Haemophilus b polysaccharide vaccine.	Polysaccharide	IM	1 dose.	1½–3½ years	Vaccination is recommended for all children at age 24 months. Routine reimmunization is not recommended but may prove useful for those immunized at an earlier age. High-risk children—those who attend day-care centers, who have traumatic or functional asplenia (eg, due to sickle cell disease), or who are immunosuppressed—may be vaccinated at age 18 months, although response to the vaccine at that age is unreliable. The vaccine can be given at the same time as DTP.
Hepatitis B	Hepatitis B vaccine (human carriers). (Recombinant DNA, produced in yeast. Human carrier–derived vaccine is no longer being manufactured in the USA but is still available commercially.)	Formalin-treated purified viral antigen	IM	2 doses 1 month apart, followed by a booster dose given 6 months later. *Do not freeze vaccine;* this causes aggregation and loss of potency. The simultaneous administration of HBIG in a separate syringe at a separate site does not appear to impair the effectiveness of the vaccine.	Variable, about 5 years	A stable, adjuvant-supplemented vaccine from highly purified formalin-inactivated HBsAg harvested from human carriers. Recommended for all individuals at high risk of exposure to HBV infection, including health care personnel such as surgeons, anesthesiologists, autopsy staff, phlebotomists, operating room and dialysis nurses, medical technologists, dentists, and other staff exposed to patients with a high rate of HBV carriage; for patients undergoing chronic hemodialysis or repeatedly receiving plasma or clotting factor concentrates for clotting disorders; in the first week of life for newborn children of carrier mothers; for sexual and household contacts of HBV carriers (families accepting children from countries with high endemic rates of HBV infection should have the child screened and should be vaccinated if the child is HBsAg-positive); for male homosexuals, intravenous drug abusers, and prison populations with problems of homosexuality and drug use; for clients and staff of institutions for the mentally retarded, and classroom contacts of aggressively behaving deinstitutionalized mentally retarded HBV carriers; for heterosexually active persons with multiple partners; for travelers to areas endemic for HBV for more than 6 months (begin series at least 6 months prior to departure), especially if sexual contacts are anticipated in these areas; and for mortlcians. Anti-HBs titers in individuals repeatedly exposed to the risk of HBV infection should be checked at intervals of 2–3 years as well as at the time of definite exposure to blood or saliva suspected of being HBsAg-positive, if not measured within the previous year. A single booster dose should be given if a nonprotective level of anti-HBs is found (see Table 37–3). There is no evidence that the vaccine can transmit acquired immunodeficiency syndrome (AIDS).

*See footnotes on p 679.

Table 37–4 (cont'd). Materials available for active immunization.*

Disease	Product (Source)	Type of Agent	Route of Administration	Primary Immunization	Duration of Effect	Comments
Influenza	Influenza virus vaccine, monovalent or bivalent (chick embryo). Composition of the vaccine is varied depending upon epidemiologic circumstances.	Killed whole or split virus types A and B	IM	1 dose. (Two doses 4 weeks or more apart are recommended in individuals who have not previously received the current antigenic components or been otherwise exposed to the current strain of virus. Two doses of the split virus products should be used in persons 12 years of age or under because of fewer side effects.)	1 year	Give immunization by November. Recommended annually for individuals with chronic cardiovascular or pulmonary disease, for residents of nursing homes and other chronic care facilities, for medical personnel who may spread infection to high-risk patients, and for healthy individuals over 65 years of age, as well as for patients with chronic metabolic disorders, renal dysfunction, anemia, immunosuppression, or asthma. Patients receiving chemotherapy for malignant disease are likely to respond better if immunized between courses of treatment.
Measles§	Measles virus vaccine, live (chick embryo).	Live virus	Subcut	1 dose at age 15 months.	Permanent	Reimmunize if given before 15 months of age; may prevent natural disease if given less than 48 hours after exposure.
Meningococcus	Meningococcal polysaccharide vaccine (combination vaccine against groups A, C, Y, and W135).	Polysaccharide	Subcut	1 dose. Since primary antibody response requires at least 5 days, antibiotic prophylaxis with rifampin (600 mg or 10 mg/kg every 12 h for 4 doses) should be given to household contacts.	?Permanent in older children and adults; transient in children < 2 years	Recommended in epidemic situations, for use by the military to prevent outbreaks in recruits, for patients with anatomic or functional asplenia, for individuals with a congenital deficiency of terminal components of the complement cascade, and possibly as an adjunct to antibiotic prophylaxis in preventing secondary cases in family contacts. Not reliably effective in infants, who require booster injections if antibody is to last for a year (especially antibody to group C). Revaccination may be indicated for individuals at high risk of infection, particularly if first immunized before age 4. The need to reimmunize adults and older children is unknown.
Mumps§	Mumps virus vaccine, live (chick embryo).	Live virus	Subcut	1 dose.	Permanent	Reimmunize if given before 1 year of age.
Pertussis	DTP.	Killed bacteria	IM	As for DTP.	See‡	Not generally recommended after the seventh birthday. Contraindications to beginning or continuing pertussis immunization include a history of seizures or the development of seizures before the 4-dose primary series is completed. Immunization of these children should be deferred until it can be determined whether an evolving neurologic illness is present. For infants who have received fewer than 3 doses of DTP, the decision to pursue immunization should be made before 1 year of age; children with seizures have a higher risk of adverse outcome from pertussis itself and are at increased risk of exposure as they grow older and contact other children. If the neurologic condition is stable and seizures are well controlled, the benefits of immunization outweigh the hazards, although the parents should be warned of an 8-fold increased risk of postimmunization convulsions if febrile convulsions have occurred previously. Definitive contraindications to further administration of DTP include hypersensitivity to the vaccine, an evolving neurologic disorder, or history of a severe reaction. The latter usually occurs within 48 hours of vaccination and is characterized by collapse in a shocklike state, persistent uncontrolled screaming for 3 hours or more, a temperature of 40.5 °C (105 °F) or higher, convulsions or a severely altered state of consciousness, general or local neurologic signs, or a systemic allergic reaction.

Disease	Product (Source)	Type of Agent	Route of Administration	Primary Immunization	Duration of Effect	Comments
Plague	Plague vaccine.	Killed bacteria	IM	3 doses 4 weeks or more apart.	6 months‡	Recommended only for occupational exposure and not for residents of endemic area in the southwest USA.
Pneumococcus	Pneumococcal polysaccharide vaccine, polyvalent.	Polysaccharide	Subcut, IM	0.5 mL, if possible before splenectomy or before instituting chemotherapy.	Uncertain—probably at least 5 years in adults, but erratic in children under age 5	Recommended for patients with cardiorespiratory disease or other chronic illness, for patients with sickle cell disease, for patients with functional, congenital, or postsurgical asplenia, and for patients with nephrotic syndrome or with cerebrospinal fluid leakage. Also suggested for immunosuppressed or alcoholic patients and for patients aged 65 or older. Only the 23 most common serotypes are incorporated in the vaccine. Children up to age 2, splenectomized children, and some chronically ill patients respond unreliably. ***Caution:*** Because of a marked increase in adverse reactions following revaccination, booster doses should generally *not* be given, even to recipients of the earlier less comprehensive and less immunogenic vaccine. However, a booster is warranted after 3–5 years for high-risk children and is recommended after 3–4 months off chemotherapy for children first immunized while receiving it. Can be given at the same time as influenza or DTP vaccines.
Poliomyelitis	Poliovirus vaccine, live, oral, trivalent (monkey kidney, human diploid).	Live virus types I, II, III	Oral	2 doses 6–8 weeks or more apart, followed by a third dose 8–12 months later. (Can be given at the same time as primary DTP immunization.) A fourth dose before entering school is recommended if the third dose was given before age 4.	Permanent	Recommended for adults only if at increased risk by travel to epidemic or highly endemic areas or occupational contact. Individuals who have completed a primary series may take a single booster dose if the risk of exposure is high.
	Poliomyelitis vaccine, inactivated.	Killed virus types I, II, III	IM	3 doses 4–8 weeks apart, followed by a fourth dose 6–12 months later. A fifth dose before entering school is recommended if the fourth dose was given before age 4. A single booster dose should be given every 5 years until age 18, after which the need is uncertain.	5 years‡	Killed virus vaccines are preferred for immunologically deficient patients and for unimmunized adults who are at risk of exposure to poliomyelitis by reason of travel or, minimally, from immunization of their children.

*See footnotes on p 679.

Table 37–4 (cont'd). Materials available for active immunization.*

Disease	Product (Source)	Type of Agent	Route of Administration	Primary Immunization	Duration of Effect	Comments
Rabies	Rabies vaccine (human diploid).	Killed virus	IM or (preexposure only) intradermal	**Preexposure:** 2 doses 1 week apart, followed by a third dose 2–3 weeks later.	2 years‡ if titer < 1:16	Preexposure immunization only for occupational or avocational risk or residence in hyperendemic area.
				Postexposure: Always give rabies immune globulin as well. (See Table 37–3.) If not previously immunized, give a total of 5 doses, on days 0, 3, 7, 14, and 28 (WHO recommends a sixth dose 90 days after the first dose). If the vaccinee is immunocompromised (or if only DEV is available), a serum specimen should be collected on day 28 or 2–3 weeks after the last dose and tested for rabies antibody.** If the antibody level is insufficient, a booster should be given and the titer remeasured 2–3 weeks later. *If previously immunized* with diploid vaccine, do not give serum therapy. Give 2 booster doses, one immediately and one 3 days later. *Note:* Unexplained immediate-type (anaphylactic) hypersensitivity reactions have occurred during primary rabies immunization with vaccine from different manufacturers. Immunization of such individuals should be discontinued unless there is actual exposure to rabies virus or inapparent or unavoidable rabies contact is truly likely to occur. In the latter situations, the serologic response to rabies should be checked and booster doses omitted if protective titers have already been attained; vaccination should be continued only under careful supervision.		For animal bite, consider antitetanus and other antibacterial measures as well. *Caution:* Several reports document unexpectedly poor response to intradermal immunization. If the intradermal route is used, rabies antibody must be measured 2–3 weeks after the third dose of vaccine. If the antibody titer is < 1:16, an additional dose should be given and the antibody level retested 2–3 weeks later. If the antibody response of an individual who has received intradermal vaccine within the past 12 months is unknown, the level should be checked; if more than 12 months have elapsed, a booster should be given and the titer tested 2–3 weeks later. If a rabies exposure takes place following preexposure immunization by the intradermal route and there is no documentation of an adequate antibody level, a full course of HRIG and IM rabies vaccine should be given. Serologic testing does not appear to be necessary if preexposure prophylaxis was given by the IM route.

Disease	Product (Source)	Type of Agent	Route of Administration	Primary Immunization	Duration of Effect	Comments
Rubella§	Rubella virus vaccine, live (human diploid).	Live virus	Subcut	1 dose (to ensure successful immunization, some experts recommend that a second dose be given to children no later than the fourth or fifth grade).	Permanent	Give after 15 months of age. Because a history of rubella is unreliable and because the vaccine is innocuous in immune recipients, unimmunized women of childbearing age should be vaccinated without serologic testing. Vaccination should be avoided during pregnancy on theoretical grounds unless there is a risk of exposure due to an outbreak; however, there is no evidence of vaccine-induced fetal damage in over 200 recipients. Vaccination is contraindicated for those receiving systemic corticosteroids for more than 2 weeks; for leukemic or immunosuppressed patients for at least 3 months after chemotherapy has been discontinued; for recipients of immune globulin treatment other than anti-Rh therapy within the following 2 weeks or prior 3 months; and for individuals with allergy to neomycin but not to eggs or penicillin. If a female is immunized postpartum and has received blood products or Rh immune globulin, serologic testing should be done 6–8 weeks later to confirm successful immunization. Viremia can occur if antibody has fallen to low levels; the frequency and thus the clinical importance of this phenomenon are unknown.
Smallpox	Smallpox vaccine (calf lymph, chick embryo). (Available from CDC‡‡).	Live vaccinia virus	Intradermal	1 dose.	3 years	Not generally available. Recommended only for laboratory workers exposed to smallpox or related poxviruses. Revaccinate if no jennerian vesicle at 6–8 days postvaccination. If patients with skin disease must be vaccinated or are exposed to a vaccinated household contact, they should receive vaccinia immune globulin. (See Table 37–3.)
Tetanus	DTP, DT (adsorbed) for children under age 7; Td, T (adsorbed) for all others.	Toxoid	IM	3 doses 4 weeks or more apart.	10 years‡**	Recipients aged 7 or above should be given a third dose 6–12 months after second. (See Table 37–3 regarding use of hyperimmune globulin.)
Tuberculosis	BCG vaccine.	Live attenuated *Mycobacterium bovis*	Intradermal, subcut	1 dose.	?Permanent††	Recommended in USA only for PPD-negative contacts of ineffectively treated or persistently untreated cases and for other unusually high risk groups.
Typhoid	Typhoid vaccine.	Killed bacteria	Subcut	2 doses 4 weeks or more apart, or 3 doses 1 week apart (less desirable).	3 years‡	70% protective. Recommended only for exposure from travel, epidemic, or household carrier and not, for example, because of floods.
Yellow fever	Yellow fever vaccine (chick embryo).	Live virus	Subcut	1 dose.	10 years†	Certificate may be required for travel. Recommended for residence in or travel to endemic areas of Africa and South America. Avoid administration to an immunologically incompetent host or an individual on long-term (>2 weeks) corticosteroid therapy.

*Dosages for the specific product, including variations for age, are best obtained from the manufacturer's package insert. Immunizations should be given by the route suggested for the product.
†Revaccination interval required by international regulations.
‡A single dose is a sufficient booster at any time after the effective duration of primary immunization has passed.
§Combination vaccines available.
**For contaminated or severe wounds, give booster if more than 5 years have elapsed since full immunization or last booster. A single booster any time after primary immunization is effective.
††Test for PPD conversion 2 months later, and reimmunize if there is no conversion.
‡‡Drug Immunobiologic and Vaccine Service, Center for Infectious Diseases. Telephone: (404) 329–3311 (main switchboard, day) or (404) 329–3644 (nights and weekends).

no defect in cell-mediated immunity usually tolerate viral infections and vaccines well but have a 10,000-fold excess of paralytic complications over the usual one case per million vaccinees, in part because of the frequent reversion of attenuated polio strains to virulence in the intestinal tract. Since live poliovirus is shed by vaccinees, it should not be given to household contacts of these patients either.

Even if administered to immunocompetent hosts, live vaccines may result in mild and, rarely, severe disease.

The early measles vaccines caused high fever and rash in a significant proportion of recipients. Subacute sclerosing panencephalitis, a rare complication of natural infection, has occurred following administration of live attenuated measles vaccine (see Chapter 32), but the rate of perhaps one case per million vaccinees is one-tenth to one-fifth the rate following natural measles, and the number of cases of measles encephalitis has fallen 100-fold since the introduction of the vaccine. The mild, recurrent arthralgia or arthritis that can follow rubella immunization may represent the consequences of a secondary rather than a primary infection in an individual with low levels of antibodies not detected by all assays and who has in vitro evidence of cell-mediated immunity.

Because passage through the human intestinal tract occasionally results in reversion of oral attenuated poliovirus vaccine (particularly type III) to neurovirulence, paralytic illness has occurred in recipients or, rarely, their nonimmune contacts, especially adults. The success of live polio vaccines in preventing widespread natural infection has resulted in the paradox that the vaccine itself now causes a large fraction of the few cases of paralytic poliomyelitis seen each year in the USA. Killed (Salk) vaccine also appears to be effective in abolishing polio, and the major advantages of live (Sabin) vaccine that sustains its use despite the small risk of paralysis (5 per million doses in nonimmune recipients) are its ease of administration and more durable immune response.

Vaccinia is not virulent for normal humans at its usual site of administration in the skin but may cause severe local illness if accidentally administered to a child with eczema or if rubbed into the eye.

Live vaccines may contain undetected and undesirable passengers. Epidemic hepatitis resulted in the past from vaccinia and yellow fever vaccines containing human serum. More recently, millions of people received SV40, a simian papovavirus, along with live or inactivated poliovirus vaccine prepared in monkey kidney tissue culture. It is disconcerting that a virus closely related to SV40 has been isolated from the brains of patients with progressive multifocal leukoencephalopathy, a lethal degenerative disease, although there is no known history of polio immunization in these cases. An increased incidence of cancer in the children of mothers who received inactivated polio vaccine during pregnancy was suggested in 2 studies but not in a 20-year follow-up of a large number of childhood recipients. SV40 can now be detected and excluded from human viral vaccines, but it is possible that presently undetected agents might be transmitted to humans with uncertain consequences, particularly by vaccines grown in nonhuman cell lines. Yellow fever vaccine has been reported to be probably contaminated with avian leukosis virus. Bacteriophages and, probably, bacterial endotoxins have also been shown to contaminate live virus vaccines, although without known hazard thus far.

Live viral vaccines probably do not interfere with tuberculin skin testing, although they depress some measurements of lymphocyte function.

Unlike live vaccines, inactivated vaccines may safely be given to immunocompromised hosts. They may not, however, dependably elicit an adequately protective immune response.

Legal Liability for Untoward Reactions

It is the physician's responsibility to inform the patient (or parent) of the risk of immunization and to employ vaccines and antisera in an appropriate manner. Certain of the risks described above are, however, currently unavoidable; the benefits almost always far outweigh the risks.

Under the current system of tort liability, manufacturers and physicians stress the risks of immunization as a means of avoiding strict product liability (liability without fault), which has been applied to the complications of immunization even when all due precautions have been taken to obtain a product that is as free of defects as the state of the manufacturing art permits; these legally motivated warnings, of course, counter public health efforts to stimulate immunization. There is some evidence that large monetary awards to statistically inevitable victims of good public health practice, some of whom may actually have suffered coincidental illness unrelated to the immunization, are leading manufacturers to abandon development and production of low-profit but medically valuable products.

The American Academy of Pediatrics and others have suggested a uniform federally administered program to resolve claims of injuries arising from publicly mandated vaccinations. Under an administrative rather than adversary process, expert panels should determine the standard of compensation for severe injuries. Compensation should cover the costs of medical care, rehabilitation, and lost wages, taking into account other sources of insurance. Punitive damages and awards for pain and suffering should be forbidden except where gross negligence or willful misconduct is demonstrated.

The cost of unpredictable untoward reactions not involving substandard manufacturing practices should logically be borne by the same public that reaps the benefits of control of disease (eg, paralytic poliomyelitis) and could be made through national compensation schemes such as exist in the United Kingdom and Japan to cover vaccine-related injuries incurred during government-recommended immunization.

Nonspecific Active Immunization

Immunization with vaccinia has been employed in attempts to nonspecifically improve the immune response and thereby decrease the frequency of recurrences of herpes labialis (cold sores). Careful evaluation has shown that this practice is ineffective—and indeed has occasionally resulted in severe illness due to uncontrolled spread of vaccinia in a patient with unsuspected immunoincompetence.

Under some circumstances, specific activation of cell-mediated immunity may lead to enhanced nonspecific ability of "activated" macrophages to deal with other antigens. Such an interaction has been demonstrated experimentally for tuberculosis, *Salmonella, Brucella, Listeria,* and *Toxoplasma* infection of animals. The apparent effectiveness of BCG immunization in the prevention of leprosy may be related to this phenomenon as well as to an antigenic similarity between *Mycobacterium tuberculosis* and *Mycobacterium leprae*. The possibility that nonspecific stimulation of the immune system with *Bordetella pertusis, Corynebacterium parvum (Propionibacterium acnes),* endotoxins, or mycobacterial products can enhance the ability of the body to reject tumor cells is being studied (see Chapter 14).

Combined Passive-Active Immunization

Passive immunization has been combined with active immunization to minimize untoward effects of certain active immunizing agents. Low-dose immune globulin (IG) decreased the side effects of the early attenuated measles vaccines, leading to greater patient acceptance. (Newer "further attenuated" vaccine strains no longer require the modifying effects of IG.) Similarly, vaccinia immune globulin decreases the likelihood of eczema vaccinatum if an eczematous patient must be vaccinated for travel to the (vanishing) smallpox-endemic area. Passive and active immunization are often simultaneously undertaken to provide both immediate but transient and slowly developing, durable protection against rabies or tetanus. The immune response to the active agent may or may not be impaired by IG if the injections are given at separate sites. Tetanus toxoid plus tetanus immune globulin may give a response superior to that generated by the toxoid alone, but after antiserum has been given for rabies, the course of immunization is usually extended to ensure an adequate response.

Parenterally administered live virus vaccines, such as measles or rubella, should not be given until at least 6, and preferably 12, weeks after the administration of IG.

Anomalously Severe Disease in the Immunized Host

Immunization may not limit the spread of infection and may sometimes contribute to the pathogenesis of the disease. Killed measles virus vaccine (no longer available) did not induce protective serum antibody but instead resulted in atypical and unusually severe rubeola upon exposure to wild virus or reimmunization with live vaccine. A poorly antigenic experimental respiratory syncytial virus vaccine increased the intensity of subsequent natural illness in infant recipients. Mice congenitally infected with lymphocytic choriomeningitis virus are clinically well until they begin to produce antibody to the virus; they then develop a fatal disorder resulting from the deposition of antigen-antibody complexes in the central nervous system and kidney. Similar poorly understood problems of intensified disease in immunized subjects have been noted with experimental trachoma and *Mycoplasma pneumoniae* vaccines.

The Decision to Immunize an Individual

Immunizing procedures are among the most effective and economical measures available for preservation and protection of health.

The decision to immunize a specific person against a specific pathogen is a complex judgment based upon an assessment of the risk of infection, the consequences of natural unmodified illness, the availability of a safe and effective immunogen, and the duration of its effect.

The organisms that cause diphtheria and tetanus are ubiquitous and the vaccines have few side effects and are highly effective, but only the immunized individual is protected. Thus, immunization must be universal.

By contrast, a nonimmune individual who resides in a community that has been well immunized against poliovirus and who does not travel has little opportunity to encounter wild (virulent) virus. Here the immunity of the "herd" protects the unimmunized person, since the intestinal tracts of recipients of oral poliovaccine fail to become colonized by or transmit wild virus. If, however, a substantial portion of the community is not immune, introduced wild virus can circulate and cause disease among the nonimmune group. Thus, focal outbreaks of poliomyelitis have occurred in religious communities objecting to immunization.

An intense debate continues over the relative risk of pertussis versus the occasional (between 1:300,000 and 1:50,000) neurologic complications of pertussis vaccination. A marked fall in pertussis immunization in the United Kingdom and Japan has been followed by a large increase in reported disease and has given greater urgency to the search for a safer vaccine.

Smallpox vaccine is effective and usually safe, but the immunity it confers is of relatively short duration, declining after about 3 years. The last known case of naturally occurring smallpox was reported from Somalia in October 1977, and the risk from even the low rate of complications significantly exceeds the benefits of vaccination. Thus, vaccination against smallpox is no longer recommended.

Previously available rabies vaccines did, if only rarely, give rise to severe reactions. The risk of exposure is low, and preexposure immunization is thus reserved for travelers to hyperendemic areas and for per-

sons with occupational or avocational hazard. Human diploid vaccine may change this risk/benefit assessment: Approximately 30,000 courses of antirabies treatment are given annually in the USA, and perhaps only 20% of these are necessary when the recommended treatment guidelines are carefully followed.

Cholera immunization offers only temporary and incomplete protection. It is of little use to travelers and should only be given where the risk of exposure is high or in fulfillment of local regulations.

Each immunologically distinct viral subtype requires a specific antigenic stimulus for effective protection. Immunization against adenovirus infection has not benefited civilian populations subject to many differing types of virus—in contrast to the demonstrated value of vaccine directed against a few epidemic adenovirus types in military recruits. Similarly, immunity to type A influenza virus is transient because of major mutations in surface chemistry of the virus every few years (antigenic shifts). These changes render previously developed vaccines obsolete and may prevent sufficient production, distribution, and utilization of new antigen in time to prevent epidemic spread of the altered strain. Major antigenic changes have been detected in visna virus recovered 1 year after experimental inoculation, suggesting a mechanism of persisting infection as well as a profound barrier to developing a successful vaccine. Antigenic variation may also be an important impediment to immunization against trypanosomes.

Age at Immunization

The natural history of a disease determines the age at which immunization is best undertaken. Pertussis, polio, and diphtheria often strike in infancy; immunization against these diseases is therefore begun shortly after birth. Serious consequences of pertussis are uncommon beyond early childhood, and pertussis vaccination is not usually recommended after 6 years of age. Since the major hazard of rubella is the congenital rubella syndrome, and since nearly half of congenital rubella occurs with the first pregnancy, it is very important to immunize as many females as possible prior to puberty. One thereby also avoids the theoretic hazard of vaccinating a pregnant female and endangering the fetus, although inadvertently immunized fetuses have thus far not been found to be damaged by their exposure to the attenuated virus.

The efficacy of immunization may also be age-related. Failure may occur because of the presence of interfering antibodies or an undeveloped responsiveness of the immune system. Infants cannot be reliably protected with live measles, mumps, or rubella vaccines until maternally derived antibody has disappeared. Because a proportion of children immunized as late as 1 year of age fail to develop antibody after measles vaccination, the age recommended for measles vaccine administration has been changed to 15 months, and some workers have made the same suggestion for rubella vaccine administration. Most of those without antibody, however, may actually have been protected, based on the lack of an IgM response to reimmunization and in vitro evidence of cell-mediated immunity. Furthermore, delay in immunization is attended by a decrease in utilization that approximates the improved rate of seroconversion. Individuals who were vaccinated at an earlier age in accordance with recommendations in effect at that time should be revaccinated. Infants frequently develop severe infections with *Haemophilus influenzae* type b, pneumococci, or meningococci, but injecting them with purified capsular polysaccharide has failed to reliably yield a good antibody response despite the excellent activity of the same antigen in older children and adults. Indeed, one study has shown that several children with early severe disease due to *H influenzae* did not develop active immunity and also failed to show a good antibody response to vaccine administered after 2 years of age. This failure to respond raises the question of a possible immune defect in the patients most in need of protection.

Technique of Immunization

When administering vaccines intended for subcutaneous or intramuscular deposition, always pull back on the syringe before depressing the plunger to make certain that the product will not be injected intravenously, with lessened immunizing effect and increased untoward reactions. It is particularly important to use a sufficiently long needle (usually > 1 inch) for intramuscular delivery of adjuvant-containing (eg, alum or aluminum phosphate-adsorbed) vaccines; the subcutaneous inoculation of adjuvants may result in tissue necrosis.

Recent studies of injection techniques suggest that the anterolateral thigh or deltoid site is preferable to the buttocks. Even with the usual precautions, use of the latter site occasionally leads to sciatic nerve damage, and, at least in adults, most injections meant for intramuscular delivery are instead delivered into fat.

The intradermal route of immunization is under intensive study as a means of obtaining an earlier or greater immune response with the same amount of antigen or of inducing a satisfactory immune response with a smaller quantity of expensive immunogens such as the hepatitis B and rabies vaccines.

Simultaneous Immunization With Multiple Antigens

Simultaneous immunization with several antigenic stimuli might be expected to result in interference by the immune response to one antigen with the development of immunity to other antigens. Actually, the simultaneous inoculation of the nonliving antigens of diphtheria, tetanus, and pertussis gives a response equal to that seen with their separate injection; the endotoxic components of *B pertussis* may even act as an adjuvant, providing a superior immune response against the additional antigens.

Similarly, the single injection of a mixture of live, attenuated measles, rubella, and mumps viruses or the simultaneous administration of live measles, small-

pox, and yellow fever vaccines gives good responses to each component of the mixture. However, between 2 and 14 days following the administration of one live virus vaccine, there is a period of suboptimal response to a **subsequently injected** live virus vaccine. Live vaccines that are not given simultaneously should be given at least 4 weeks apart if time permits. The administration of cholera and yellow fever vaccines within 1–3 weeks of each other decreases the antibody response to both agents. These immunizations also should therefore be given at the same time or at least 4 weeks apart. Finally, it is always best to administer multiple immunizing agents according to a schedule that has been demonstrated to yield an effective response.

Recommendations for Childhood Immunization

Despite the extraordinary impact of immunization in the developed world, WHO estimates that of every 1000 children born today, 5 are crippled by poliomyelitis, 10 die of neonatal tetanus, 20 die of pertussis, and 30 more die of measles and its complications. A rational program of immunization against infectious diseases begins in childhood, when many of the most damaging and most preventable infections normally appear. Table 37–5 summarizes the current guidelines for immunization in childhood as compiled by the Expert Committee on Infectious Diseases of the American Academy of Pediatrics. The need for childhood immunization has increased, because unimmunized individuals in a partially immune population will be less exposed to and will therefore develop later than they otherwise would such typically childhood diseases as measles and mumps. When these illnesses do occur in adolescence or adulthood, they are often much more severe than in childhood as well as being diagnostically bewildering to the physician unprepared for such illnesses in this age group. Thus, epidemic measles now occurs in college students. Where school attendance requirements mandate immunization, the rates are high, eg, over 95% at school entry in a survey of 1982–1983.

The physician should provide the patient with a clear and up-to-date record of all immunizations, which is useful in future medical encounters and in fulfilling school registration and other institutional requirements. Physicians can improve immunization rates by developing recall systems to identify children who are due for immunizations and by using the occasion of a visit for intercurrent illness to investigate and augment a patient's immune status. It is *not* necessary to restart an interrupted series of vaccinations or to add extra doses. If the vaccination history is unknown and there are no obvious contraindications, the child or adult should be fully immunized appropriately for age. Reimmunization poses no significant risk.

For developing nations in which much preventable serious infection occurs in the first few years of life, WHO recommends an accelerated immunization program: at birth, oral poliovirus and BCG; at ages 6, 10, and 14 weeks, oral poliovirus and DTP; at age 9 months, measles (to be repeated in the second year of life if given before age 9 months).

Immunization of the Elderly

The 3 most important vaccinations for the elderly are those against influenza, pneumococcal infections, and tetanus-diphtheria. Fewer than 30% of the elderly receive influenza vaccine, although it is generally effective and has been recommended for many years. Fewer still receive pneumococcal vaccine, which is thought to be cost-effective despite the absence of conclusive controlled trials in this age group. The highest incidence of tetanus in the USA is in persons over 60 years of age, half of whom show low antitoxin levels. Hospitalization provides an excellent opportunity to vaccinate this group.

Immunization for Foreign Travel

National health authorities may require an International Certificate of Vaccination against cholera or yellow fever from travelers, usually depending upon the presence of these diseases in countries on their itinerary. Cholera vaccinations may be given by any licensed physician; because it is not very effective, it is not generally recommended except where required. The certificate must be completed in all details and then validated with an approved stamp. Yellow fever vaccination may only be administered and the certificate validated at an officially designated center (these may be located by contacting the state or local health department). In addition to these legal requirements, all adults are advised to be adequately immunized against measles, tetanus, and diphtheria and to undergo additional immunizations (poliomyelitis, typhoid, hepatitis A, meningococcus) if visiting areas where the frequency of illness in the population or the

Table 37–5. Guidelines for routine immunization of normal infants and children.

Disease	Vaccine	Schedule of Doses				
		First	Second	Third	Fourth	Fifth
Diphtheria-tetanus-pertussis	DTP, adsorbed	2 months	4 months	6 months	1½ years	4–6 years*
Poliovirus I, II, and III	Oral trivalent	2 months	4 months	6 months†	1½ years	4–6 years‡
Measles-mumps-rubella	MMR or singly	15 months	...	...	...	...

*Adult-type combined tetanus-diphtheria toxoid (Td) is recommended at 10-year intervals thereafter.

†Optional in nonendemic areas.

‡Only 3 doses are necessary if the third dose is given after the fourth birthday.

level of sanitation increases the risk of infection. Travelers should be immunized against plague if contact with wild rodents or rabbits in an endemic rural area is anticipated, and to hepatitis B if sexual contacts are anticipated in Southeast Asia or sub-Saharan Africa. A formalin-inactivated vaccine against Japanese B encephalitis, not licensed in the USA, may be available in several Asian countries where the infection is endemic; long-term travelers to areas with a significant risk of infection (China, India, Japan, Korea, Nepal, and Thailand) should ask at their embassies how vaccine may be obtained. (Travelers to malaria-endemic areas should also be advised regarding chemoprophylaxis.) Information regarding individual agents may be found in Tables 37–3 and 37–4.

Smallpox vaccination is no longer required for international travel and is in fact contraindicated.

No special immunizations are generally recommended for persons traveling from the USA to Western Europe, Canada, Australia, or Japan. Detailed suggestions of the USPHS are given country by country in its Health Information for International Travel Supplement (see references).

Vaccines & Antisera of Restricted Availability or Experimental Status

Recombinant DNA technology is being applied to production of vaccines against hepatitis B, herpes simplex virus infection, malaria, and gonorrhea. The production of anti-idiotype antibodies may provide nontoxic or nonpathogenic immunogens capable of inducing both humoral and cellular immunity, even where the structure of the antigen has yet to be determined.

A number of vaccines are available for individuals at greatly increased occupational risk but not for the general public. Only a partial listing will be given here.

Adenovirus. Live attenuated oral vaccines have been developed for military use. These are directed against the 2 types of virus—types 4 and 7—that commonly cause severe epidemic disease in recruits. Experimental vaccines have been formulated against additional (civilian) serotypes.

Adjuvants. At least 3 promising synthetic compounds are under active evaluation: muramyl dipeptides, polynucleotides, and liposomes. Adjuvant compounds may allow immunization of infants who respond poorly to the polysaccharide antigens of *H influenzae*, pneumococci, and meningococci.

AIDS. The failure of patients with AIDS to form antibody to neoantigens suggests the possible use of prophylactic immune globulin in this disease, but the clinically dominant pathogens in these patients are those that the body combats chiefly by cell-mediated immune mechanisms.

Anthrax. A protein antigen extracted from culture filtrates can protect those who work with imported animal hides and hair and others with occupational exposure.

Arbovirus (various). Vaccines against certain agents causing equine encephalitis are available for persons working with the viruses. Experimental vaccines, either formalin-inactivated or related live, avirulent strains, protect animals against clinically important hemorrhagic fevers of Africa and Argentina, and immune plasma may have therapeutic utility. A live attenuated dengue type 2 virus vaccine protects monkeys against experimental infection but gives a high rate of severe reactions in human volunteers.

Bacteroides fragilis. Rats immunized with capsular polysaccharide of this common participant in intra-abdominal sepsis do not develop early bacteremia or form abscesses after intraperitoneal inoculation of *B fragilis* or *Bacteroides distasonis*.

Campylobacter. Because of partial immunologic cross-reaction, experimental toxoids directed against the heat-labile toxins of *Escherichia coli* and *Vibrio cholerae* may protect against infection with *Campylobacter jejuni*, as well.

Cholera. The low level of immunity induced by commercial phenol-killed whole-cell vaccines has led to the development of experimental vaccines employing formalin-killed or live, attenuated bacteria and a largely detoxified yet immunogenic "procholeragenoid." Oral immunization may be more effective than parenteral.

Clostridia. A toxoid prepared from the beta toxin of *Clostridium perfringens* type C (pig-bel vaccine) successfully prevents this necrotizing enterocolitis of Papua New Guinea, although antitoxin given at the time of acute illness is ineffective.

Cytomegalovirus. A live attenuated vaccine grown in a human diploid cell line has been shown to be immunogenic when given subcutaneously but not intranasally. The safety and efficacy of the vaccine, in view of the propensity of herpesviruses to cause latent infections which give rise to disease at the time of reactivation, is of concern. The administration of the Towne strain vaccine prior to renal transplantation did not result in reactivation of infection with the vaccine strain following surgery, but serologic responses were poorer than in normal recipients and many patients did not develop cell-mediated immunity to CMV. The vaccine does not alter lymphocyte subsets ratios in normal volunteers. There is evidence that prior infection (or immunization) may modify subsequent disease; clinically apparent neonatal infection and death from cytomegalovirus is less frequent among the children of highly seroimmune mothers than in those of a less immune group. On the other hand, patients who demonstrate lymphocyte transformation to herpesviruses lose these responses after transplantation, and live virus immunization before transplantation may be no more effective in preventing or modifying infection than natural immunity. Indeed, in one small uncontrolled trial, immunized renal transplant patients developed exogenous cytomegalovirus infections at the same rate as that previously reported in unimmunized patients. The vaccine did not prevent viral excretion or clinical illness in recipients who were already seropositive prior to immunization; symptoms

were substantially milder in those vaccinated, however. These patients may benefit from passive immunization. High-titer and anti-cytomegalovirus plasma given prophylactically to recipients of bone marrow transplants appears to decrease symptomatic infections, including interstitial pneumonia. Plasma is less effective in the face of granulocyte transfusion. It has been reported to abate the persistent fever of established infection in recipients of renal transplants.

Dental caries. Subcutaneous administration of a purified protein from *Streptococcus mutans* to primates resulted in a 70% decrease in caries and a fall in the numbers of streptococci recovered from dental plaque. Decreased colonization also follows oral administration of killed whole cells in humans, and extensive trials in humans are planned.

Echovirus. Administration of IgG has been reported to prevent infection due to echovirus during an outbreak in a neonatal intensive care unit and to ameliorate encephalitis in a patient with X-linked hypogammaglobulinemia; this finding is consistent with clinical observations of hypersusceptibility of agammaglobulinemic patients to this virus.

***Escherichia coli*, enterotoxigenic.** Experimental animals achieve greatest protection when immunized against both toxin and colonization factor. The amino acid sequences of colonization factors are being worked out to facilitate vaccine production, but suppression of colonization by one serotype has led to an increased prevalence of another potentially pathogenic strain. The neutralizing antibody response to both heat-labile and heat-stable toxins is being improved. Heat-labile toxins of all strains are antigenically similar and related to the cholera toxin; heat-stable toxins are also often related serologically, although at least 2 types of the latter are known. Synthetic heat-stable toxin coupled with the nontoxic B subunit of heat-labile toxin yields a vaccine that protects rats and rabbits against challenge with heterologous strains producing either toxin. Oral booster doses of vaccine must avoid degradation of heat-labile toxin by gastric acid. Homologous protection against oral reinfection has also been demonstrated in human volunteers who have recovered from diarrheal illness.

***Escherichia coli* K1 neonatal meningitis.** Two-thirds of cases of neonatal meningitis are due to *E coli* strains, and more than 80% of these strains carry the K1 capsular polysaccharide antigen. Pilus attachment appears important for colonization by K1 strains; an antipilus vaccine might prevent adherence and subsequent invasion. It might be necessary to immunize the mother in order to prevent neonatal infection. Rats that have been suckled by K1-immunized mothers or given oral antiserum to *E coli* K1 have less bacteremia and lower mortality rates following intraperitoneal instillation of *E coli* K1, suggesting a means of preventing and possibly even treating this severe neonatal illness.

Gonococci. Although experimental vaccines have been shown to produce antibody, the role of immunity in protecting against gonococcal infection is not known at this time. A number of differing gonococcal immunotypes have been identified, consistent with the clinical experience of multiple episodes of illness in an individual and suggesting that vaccine development will be difficult. Current attempts to identify common fimbrial antigens are aimed at producing antiadhesins that would prevent attachment of multiple serotypes.

Gram-negative bacteremia. Vaccines directed against the somatic O antigens of gram-negative bacteria are partially protective against infection with these organisms—for example, pertussis, cholera, plague, and typhoid vaccines. The antigenic heterogeneity of the many serotypes of different species has been thought to preclude a general "anti-O" vaccine, although uncontrolled trials suggest that pooling anti-LPS immunoglobulin may provide a sufficiently broad antimicrobial spectrum. In one study, over half of 234 invasive strains of *E coli*—the single most common cause of bacteremia—shared eleven O, six K, and nine H antigens. The endotoxic lipopolysaccharides (LPS) of nearly all gram-negative species share a common "core" antigen. Serum from volunteers hyperimmunized with a core (J5) vaccine was administered to patients with gram-negative bacteremia and nearly halved the mortality rate in one double-blind controlled trial, but efficacy was unclear in another trial, suggesting a need for more frequent dosing or for an intravenous preparation of IgM antibody, which may be a superior antiendotoxin. Specific "anti-O" vaccines may decrease the extent of renal damage in ineradicable chronic urinary infections.

***Haemophilus influenzae* type b.** Efforts continue to improve upon the licensed polyribophosphate vaccine, which does not induce protective antibody in infants. Alternatives under investigation include administration of the capsular antigen with DTP or conjugated to tetanus toxoid to obtain an adjuvant effect; protection by bactericidal antibody induced to common outer membrane proteins; prevention of bacterial adherence with antibody directed to a common pilus antigen or the common IgA-protease; and injection of hyperimmune serum or monoclonal antibodies to improve the outcome of established infections.

Hepatitis A. A vaccine grown in diploid cell culture is being tested.

Hepatitis B. Heat inactivation may preserve antigenicity better than formalin, in view of the better responses to the Netherlands Red Cross vaccine in dialysis patients than with the Merck and Merieux products. Synthetic antigens are also being evaluated.

Herpesvirus hominis. Exogenous reinfection with a different strain of the same serotype has been clearly documented. Formalinized vaccine has failed to yield protection in 2 separate human trials despite success in animal models. Immunosuppressed recipients of renal transplants, whose cell-mediated immunity is impaired, may develop severe herpetic disease despite very high levels of antibody. It may be that vaccine can prevent primary infection in nonimmune sexual partners of individuals with genital infection or when administered in early childhood. A type 2 glycoprotein

subunit vaccine has induced cell-mediated immunity lasting for more than 7 months.

Influenza. Live attenuated vaccines, including mutant strains that replicate poorly at deep body temperature (ts strains), have been examined as potentially superior immunizing agents compared to currently licensed killed vaccines. The ts strains give better nasal than serum antibody responses, but human ts strains have tended to revert to virulence, and research now focuses upon attenuated reassortant vaccines based upon avian isolates. Intranasal immunization with live virus is attractive as a means of improving the local barriers to initiating infection, but minor decreases in pulmonary function are detected from 1 to 3 weeks thereafter, and the hazard to subjects with damaged lungs has not been defined. Other approaches being examined include the use in Russia of orally administered live attenuated strains and the use of recombinant viruses containing only new viral neuraminidase antigens. The latter vaccine is less protective than a vaccine inducing antibody against the new viral hemagglutinin but permits colonization and active immunization by asymptomatic or clinically attenuated illness.

Kawasaki disease. In a controlled trial, intravenous immunoglobulin appeared to prevent coronary aneurysms.

Klebsiella. A polysaccharide-containing vaccine protects against experimental burn wound sepsis. The heat-stable toxin from a strain associated with diarrhea shares the structure of the heat-stable toxin of *E coli*.

Legionella. Avirulent strains are readily produced on subculture and appear to be effective immunizing agents in guinea pigs.

Leprosy. Immunogenic, heat-killed *Mycobacterium leprae* will be tested in humans with and without concomitant BCG.

Malaria. Although vaccination with killed merozoites in Freund's adjuvant has been reported to protect humans against *Plasmodium falciparum* and although the asexual erythrocytic forms of the parasite have been cultivated in vitro, numerous barriers to a successful malaria vaccine remain. *P falciparum* exhibits marked antigenic heterogeneity, but the need to retain functionality in the face of genetic diversity may result in a conserved (common) receptor antigen on the merozoite for attachment to red blood cells or on parasitized red cells for adherence to postcapillary venules, the obstruction of which causes most of the clinical findings in malaria. More immediate promise may lie with inducing immunity to the sporozoites inoculated by the biting mosquito. Unfortunately, malaria infection is itself immunosuppressive and has blocked the anamnestic response expected from prechallenge immunization with an irradiated sporozoite vaccine. Suitable safe adjuvants are not available, but in the absence of adjuvants, vaccine responses are transient. Recombinant DNA techniques may be necessary to produce sufficient immunizing materials, although it is now possible to cultivate *P falciparum* in human hepatocytes.

Measles. A live vaccine grown in diploid cells protects children at age 4–6 months and offers a promising means of mass immunization in developing countries where the death rate from early measles is high. Immunization at this young age may not be necessary, however, since vaccination at age 9 months appears to halt transmission of measles to younger children. A heat-stable strain is expected to greatly improve vaccine distribution. A campaign to eradicate measles worldwide, as was done for smallpox, has been proposed.

Meningococcus. The protection-inducing antigen for group B is a type-specific protein, not a group-defining polysaccharide, as in groups A and C. Infants respond poorly to meningococcal and other polysaccharides, but immunization with polysaccharide coupled to tetanus toxoid may be more effective. The group B polysaccharide, a poor immunogen in all age groups, elicits bactericidal IgM antibody when coupled to outer membrane proteins.

Mycoplasma pneumoniae. Early killed vaccine may have caused more severe disease in the immunized host. Live, temperature-sensitive mutants that cannot multiply at the temperature of the lower respiratory tract induce protection against experimental vaccination of animals has shown poor correlation of complement-fixing antibody and protection. Since low levels of mycoplasmacidal antibody are found in a high proportion of children below the age of 5, and since repeat attacks of mycoplasmal pneumonia are well documented, it would appear that antibody is not protective against this infection or that immunity is particularly short-lived, making the long-term value of immunization uncertain. Experimental studies in mice suggest that persistence of intranasal immunity may be more important than serum antibody levels.

Pertussis. Respiratory challenge has been suggested as a more reliable test of efficacy than the standard assay employing intracerebral inoculation of mice. A purified hemagglutinin component vaccine developed and widely used in Japan is less toxic than other current products but may be deficient in protective capacity. The Japanese vaccine lacks one of the 3 major serotype antigens, a deficiency that led to earlier vaccine failures in several European countries. The new vaccine will thus require extensive proof of efficacy before it is widely adopted.

Pneumococcus. Temperature-sensitive noninvasive mutants capable of colonizing the upper airways are being studied as possible immunogens, as are antigenically related viridans streptococci. Immunogenicity is improved by coupling the polysaccharide antigens to proteins such as diphtheria or tetanus toxoids. A semisynthetic vaccine incorporated into liposomes is also being evaluated; it gives an IgM antibody response that in animals is a far more effective opsonin than IgG. Hyperimmune antipneumococcal IgG from vaccinated volunteers is being evaluated for the treatment of established infection.

Pseudomonas aeruginosa. Polyvalent lipopolysaccharide vaccines can stimulate the development of

protective opsonizing antibodies, but clinical usefulness in immunosuppressed, neutropenic patients or in patients with cystic fibrosis, 2 of the groups most at risk from this organism, has not been demonstrated. Vaccination may protect the patient with normal humoral immunity and normal white cell count against bacteremia from infected burns. Side reactions to immunization are frequent. Serotype-specific polysaccharide antigens, ribosomal preparations, and toxoids of exotoxin A appear less effective than anti-LPS.

Pseudomonas pseudomallei. An antipolysaccharide vaccine protects against experimental infection and may be particularly useful in protecting hooved animals of Southeast Asia from this ubiquitous environmental pathogen.

Rabies. Intradermal immunization has been reported to provide an earlier antibody response than the intramuscular route recommended for postexposure prophylaxis, ultimately giving the antibody levels reached with intramuscular vaccination but using only 25–30% as much of this expensive immunogen. The intradermal route is unlikely to be widely adopted until an explanation is found for the generally poor immune responses to intradermal preexposure prophylaxis that were discovered after an immunized Peace Corps worker in Africa died of rabies. Potential factors in this vaccine failure included problems in vaccine storage, simultaneous antimalarial prophylaxis, and multiple immunizations. Both French and American vaccines administered intramuscularly have subsequently performed poorly in at least some trials; repeated problems with the American vaccine led to its withdrawal.

Respiratory syncytial (RS) virus. Recipients of early killed vaccines developed more serious illness than unimmunized infants. Encouragingly, however, monoclonal antibodies to the viral fusion protein have completely protected mice from viral challenge without any evidence of clinical or histologic deterioration. A live attenuated virus vaccine produced antibody in most recipients over 1 year of age, suggesting interference from persisting maternal antibody, but failed to protect against clinical illness. The nasal route of immunization may prove more effective in the presence of serum antibody.

Rhinovirus. Live and inactivated vaccines have been produced. Their use does not appear promising at present because of the multiplicity of serotypes that would be needed and because even natural immunity offers only partial protection.

Rickettsioses. A formalin-inactivated **Q fever** vaccine, produced in chick embryo cell culture, is undergoing clinical trials in humans. The vaccine, produced by the US Army Institute of Infectious Diseases at Fort Detrick, Maryland, could be particularly valuable for animal handlers and researchers utilizing pregnant sheep in experimental surgery, a group with a relatively high incidence of occupationally acquired infection. Skin or lymphocyte activation testing is more reliable than measurement of complement-fixing antibody in determining immunity to Q fever and should be employed to screen out individuals who will have severe local reactions to this vaccine.

A similar investigational vaccine, also produced at Fort Detrick, protected monkeys but not human volunteers against challenge with **Rocky Mountain spotted fever.** If cell-mediated immunity is more important than antibody in protection against this infection, a live attenuated vaccine may be more effective.

Antigenic heterogeneity hampers the effectiveness of killed vaccines for **scrub typhus.**

Rotavirus. The 4 human serotypes of this common agent are probably a major cause of infant death in developing countries. Oral immune globulin may alleviate infection and symptoms if given prophylactically to low-birth-weight infants in an endemically infected environment but does not alter the symptoms of established infection. A live attenuated bovine strain vaccine, which is labile at acid pH, has protected infants during an outbreak.

Shigella. Live attenuated oral vaccines utilizing either noninvasive hybrid *E coli* modified to carry *Shigella* antigens or streptomycin-dependent shigellae are being evaluated. Lack of immunogenicity and reversion to virulence, respectively, have been problems in the development of these 2 vaccines. Incorporation of a *Shigella sonnei* plasmid in an experimental typhoid vaccine has resulted in protection of mice against both pathogens and has been safely administered to human volunteers.

***Streptococcus* group A.** It is now possible to protect human volunteers with type-specific M protein determinants free of contamination with nonspecific antigens. The purified materials do not cause local and systemic reactions, nor do they lead to the production of antibodies reactive with the heart, as did earlier vaccines.

***Streptococcus* group B.** The commonest agent of neonatal meningitis colonizes the female genital tract and causes disease in the absence of transplacentally shared maternal antibody directed against the type-specific antigens. Purified capsular polysaccharides induce antibody responses in nonimmune female volunteers without adverse effects and could be given to the mother to ensure adequate antibody levels in the neonate at delivery. To avoid the possibility of immunologic paralysis of the fetus, it might be necessary to immunize women of childbearing age before pregnancy. However, protective antibody levels in the mother do not always result in protective levels in cord sera, particularly in the premature infants most at risk of disease; the ratio of cord to maternal IgG is about 1 at 40 weeks but is 0.5 at 32 weeks and only 0.33 at 28 weeks. The use of hyperimmune serum to combat established infection is also under study.

Syphilis. Repeated intravenous injections of gamma ray-irradiated motile treponemes protect against experimental intradermal infection for at least 1 year but are impractical. Fewer injections using stored treponemes gave only partial protection. Immune serum protects hamsters even when given 1 week after exposure to infection but does not alter established infection. Immunogenic *T pallidum* antigens

have been produced in *E coli*.

Trachoma. Vaccination with irradiated *Chlamydia trachomatis* organisms induces antibody but increases the severity of subsequent infection.

Tularemia. A live attenuated vaccine for intradermal administration, developed at Fort Detrick, Maryland, produces long-lasting humoral and cell-mediated immune responses.

Typhoid. Live oral attenuated mutants provide over 90% protection for at least 3 years and decrease enteric carriage of the infective organism, with no important side effects. One vaccine strain has been modified to incorporate protection against infection with *S sonnei*. Vi polysaccharide, covalently bound to a carrier protein to generate a T-dependent antigen, is also under evaluation.

Varicella. A live attenuated vaccine is highly effective in normal children and is well tolerated in immunosuppressed children if chemotherapy is discontinued 1 week or more prior to vaccination. Although the vaccine is only 80% effective in blocking all manifestations of disease in the latter group, severe disease is almost completely prevented. Vaccination of immunosuppressed children may not offer durable immunity, however. Vaccine virus does not spread readily to contacts and induces immunity for at least 7–10 years in 95% of recipients, but it is capable of latency and has been isolated from lesions of herpes zoster. Latency may be a virtue and help to sustain the level of immunity so that severe disease due to wild-type virus does not arise in adult years if and when vaccine-induced immunity wanes. It has been suggested that the elderly, in whom natural cell-mediated immunity to varicella-zoster virus often *has* diminished, be given a vaccine booster to prevent herpes zoster. The vaccine virus is susceptible to acyclovir.

IMMUNIZATION AGAINST NONINFECTIOUS DISEASES

Prevention of Rh Isoimmunization

Rh-negative females who have not already developed anti-Rh antibodies should receive Rh immune globulin within 72 hours after obstetric delivery, abortion, accidental transfusion with Rh-positive blood, chorionic villus biopsy, and, probably, amniocentesis, especially if the needle passes through the placenta. This passive immunization suppresses the mother's normal immune response to any Rh-positive fetal cells that may enter her circulation, thus avoiding erythroblastosis fetalis in future Rh-positive fetuses; it may protect in a nonspecific manner as well, analogous to the "blocking" effect of high-dose IgG in ameliorating autoimmune diseases such as idiopathic thrombocytopenic purpura. Even if more than 72 hours has elapsed after exposure, the globulin should be administered, since it will be effective in at least some cases. Three of 6 subjects were protected from the immunogenic effect of 1 mL of Rh-positive red cells given intravenously by 100 μg of anti-Rh globulin given *13 days later*. Some workers have also suggested the administration of anti-Rh globulin to Rh-negative newborn female offspring of Rh-positive to prevent possible sensitization from maternal-fetal transfusion (see Chapters 22 and 34).

A significant number of Rh isoimmunizations occur during pregnancy rather than at the time of delivery. This sensitization can be almost completely prevented by administration of anti-Rh globulin at 28 and 34 weeks of gestation, despite the theoretic risk of the globulin crossing the placenta and causing erythroblastosis in the fetus.

There is disagreement about whether or not antenatal Rh prophylaxis should be widely adopted, because of its marginal improvement on rate of sensitization despite at least a 4-fold increase in costs, the unknown hazards to the fetus of administering other unwanted antibodies, and the strain upon supplies of the Rh immune globulin, with unknown risks to hyperimmunized donors. The last problem may be resolved by the reported cloning of an anti-D cell line and production of a monoclonal antibody. There is also the prospect that the combination of plasma exchange and "blocking" with high-dose intravenous Ig in the last trimester can suppress the level of Rh antibodies in an already sensitized female and prevent erythroblastosis in the Rh-positive fetus.

Prevention of Diabetes

It is strongly suspected that juvenile-onset diabetes mellitus is often induced by a viral infection, most likely coxsackievirus B4 or mumps virus, particularly in genetically-predisposed individuals. If further study confirms this or a similar etiologic relationship, it may be possible for the first time to prevent a chronic progressive noninfectious disease through anti-infective immunization.

Serum Therapy of Poisonous Bites

The toxicity of the bite of the black widow spider, the coral snake, and crotalid snakes (rattlesnakes and other pit vipers) may be lessened by the administration of commercially available antivenins.

Antisera for scorpion stings and rarer poisonous bites, especially of species foreign to North America, may also be available.

Information on the use and availability of antivenins is often available from Poison Control Centers, particularly those in cities having large zoos, such as New York and San Diego. A Snakebite Trauma Center has been established at Jacobi Hospital in New York ([212] 430–8183) and cooperates with the Bronx Zoo Reptile House, a repository of many exotic species ([212] 220–5042; after 5 PM, [212] 430–6494). In addition, an antivenin index listing the availability of all such products is maintained by the Poison Control Center in Tucson, Arizona ([602] 626–6016 or 626–6000).

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Appendix

Glossary of Terms Commonly Used in Immunology

Abrin: A potent toxin which is derived from the seeds of the jequirity plant and which agglutinates red cells (a lectin).

Absolute catabolic rate: The mass of protein catabolized per day, which is determined by multiplying the fractional turnover rate by the volume of the plasma pool.

Accessory cells: Lymphoid cells predominantly of the monocyte and macrophage lineage that cooperate with T and B lymphocytes in the formation of antibody and in other immune reactions.

Active immunity: Protection acquired by deliberate introduction of an antigen into a responsive host.

Activated lymphocytes: Lymphocytes that have been stimulated by specific antigen or nonspecific mitogen.

Activated macrophages: Mature macrophages in a metabolic state caused by various stimuli, especially phagocytosis or lymphokine activity.

Activation: A process in which the members of the complement sequence are altered enzymatically to become functionally active.

Adenosine deaminase: An enzyme that catalyzes the conversion of adenosine to inosine and is deficient in some patients with combined immunodeficiency syndrome.

Adjuvant: A compound capable of potentiating an immune response.

Adoptive transfer: The transfer of immunity by immunocompetent cells from one animal to another.

Adrenergic receptors: Receptors for various adrenergic agents of either the α or the β class that are present on a variety of cells and from which the action of various adrenergic drugs can be predicted.

Affinity chromatography: A technique in which a substance with a selective binding affinity is coupled to an insoluble matrix such as dextran and binds its complementary substances from a mixture in solution or suspension.

Agammaglobulinemia: See **Hypogammaglobulinemia.**

Agglutination: An antigen-antibody reaction in which a solid or particulate antigen forms a lattice with a soluble antibody. In reverse agglutination, the antibody is attached to a solid particle and is agglutinated by insoluble antigen.

Alexin: (also **alexine**): A term coined by Pfeiffer to denote a thermolabile and nonspecific factor that, in concert with sensitizer, causes bacteriolysis.

Allele: One of 2 genes controlling a particular characteristic present at a locus.

Allelic exclusion: The phenotypic expression of a single allele in cells containing 2 different alleles for that genetic locus.

Allergens: Antigens that give rise to allergic sensitization by IgE antibody.

Allergoids: Chemically modified allergens that give rise to antibody of the IgG but not IgE class, thereby reducing allergic symptoms.

Allergy: An altered state of immune reactivity, usually denoting hypersensitivity.

Allogeneic: Denotes the relationship that exists between genetically dissimilar members of the same species.

Allogeneic effect: A form of general immunopotentiation in which specific stimulation of T cells results in the release of factors active in the immune response.

Allograft: (also **homograft**): A tissue or organ graft between 2 genetically dissimilar members of the same species.

Allotype: The genetically determined antigenic difference in serum proteins, varying in different members of the same species.

Alpha-fetoprotein (AFP): An embryonic α-globulin with immunosuppressive properties that is structurally similar to albumin.

Alternative complement pathway (also **properdin pathway**): The system of activation of the complement pathway through involvement of properdin factor D, properdin factor B, and C3b, finally activating C3 and then progressing as in the classic pathway.

Am marker: The allotypic determinant on the heavy chain of human IgA.

Amboceptor: A term coined by Ehrlich to denote a bacteriolytic substance in serum that acts together with complement or alexin, ie, antibody.

Anamnesis (also **immunologic memory**): A heightened responsiveness to the second or subsequent administration of antigen to an immune animal.

Anaphylactoid reaction: Clinical response similar to anaphylaxis but not caused by antigen-antibody reaction.

Anaphylatoxin: A substance produced by complement activation that results in increased vascular permeability through the release of pharmacologically active mediators from mast cells.

Anaphylatoxin inactivator: An α-globulin with a molecular weight of 300,000 that destroys the biologic activity of C3a and C5a.

Anaphylaxis: A reaction of immediate hypersensitivity present in nearly all vertebrates that results from sensitization of tissue-fixed mast cells by cytotropic antibodies following exposure to antigen.

Anergy: The inability to react to a battery of common skin test antigens.

Angiogenesis factor: Released by macrophages and causes neovascularization of surrounding tissues.

Antibody: A protein which is produced as a result of the in-

troduction of an antigen and which has the ability to combine with the antigen that stimulated its production.

Antibody combining site: That configuration present on an antibody molecule which links with a corresponding antigenic determinant.

Antibody-dependent cell-mediated cytotoxicity (ADCC): A form of lymphocyte-mediated cytotoxicity in which an effector cell kills an antibody-coated target cell, presumably by recognition of the Fc region of the cell-bound antibody through an Fc receptor present on the effector lymphocyte.

Antigen: A substance that can induce a detectable immune response when introduced into an animal.

Antigen processing: The series of events that occurs following antigen administration and antibody production.

Antigen-binding site: The part of an immunoglobulin that binds antigen.

Antigenic competition: The suppression of the immune response to 2 closely related antigens when they are injected simultaneously.

Antigenic determinant (see also **Epitope**): That area of an antigen which determines the specificity of the antigen-antibody reaction.

Antigenic modulation: The spatial alteration of the arrangement of antigenic sites present on a cell surface brought about by the presence of bound antibody.

Antiglobulin test (Coombs test): A technique for detecting cell-bound immunoglobulin. In the direct Coombs test, red blood cells taken directly from a sensitized individual are agglutinated by antigammaglobulin antibodies. In the indirect Coombs test, a patient's serum is incubated with test red blood cells and the sensitized cells are then agglutinated with an anti-immunoglobulin or with Coombs reagent.

Antilymphocyte serum: Antibodies which are directed against lymphocytes and which usually cause immunosuppression.

Antinuclear antibodies (ANA): Antibodies which are directed against nuclear constituents, usually in nucleoprotein, and which are present in various rheumatoid diseases, particularly systemic lupus erythematosus.

Antitoxins: Protective antibodies that inactivate soluble toxic protein products of bacteria.

Apheresis: Process of removing blood or a blood element from the body.

Armed macrophages: Macrophages capable of antigen-specific cytotoxicity as a result of cytophilic antibodies or arming factors from T cells.

Arthus phenomenon: A local necrotic lesion resulting from a local antigen-antibody reaction and produced by injecting antigen into a previously immunized animal.

Association constant (K value): The mathematical representation of the affinity of binding between antigen and antibody.

Atopy: A genetically determined abnormal state of hypersensitivity as distinguished from hypersensitivity responses in normal individuals, which are not genetically determined.

Attenuated: Rendered less virulent.

Autoantibody: Antibody to self antigens.

Autoantigens: Self antigens.

Autograft: A tissue graft between genetically identical members of the same species.

Autoimmunity: Immunity to self antigens (autoantigens).

Autoradiography: A technique for detecting radioactive isotopes in which a tissue section containing radioactivity is overlaid with x-ray or photographic film on which the emissions are recorded.

B cell (also **B lymphocyte**): Strictly, a bursa-derived cell in avian species and, by analogy, a bursa-equivalent derived cell in nonavian species. B cells are the precursors of plasma cells that produce antibody.

Bacteriolysin: An antibody or other substance capable of lysing bacteria.

Bacteriolysis: The disintegration of bacteria induced by antibody and complement in the absence of cells.

Baseline cellular phagocytosis: Digestion by phagocytic cells and effector mechanisms that have developed for dealing with potential invading pathogens.

BCG (bacillus Calmette-Guérin): A viable attenuated strain of *Mycobacterium bovis* which has been obtained by progressive reduction of virulence and which confers immunity to mycobacterial infection and possibly possesses anti-cancer activity in selected diseases.

Bence Jones proteins: Monoclonal light chains present in the urine of patients with paraproteinemic disorders.

Beta lysin: A highly reactive heat-stable cationic protein that is bactericidal for gram-positive organisms.

Biosynthesis: The production of molecules by viable cells in culture.

Blast cell: A large lymphocyte or other immature cell containing a nucleus with loosely packed chromatin, a large nucleolus, and a large amount of cytoplasm with numerous polyribosomes.

Blast transformation: See **Lymphocyte activation.**

Blocking antibody: See **Blocking factors.**

Blocking factors: Substances that are present in the serum of tumor-bearing animals and are capable of blocking the ability of immune lymphocytes to kill tumor cells.

Bradykinin: A 9-amino-acid peptide which is split by the enzyme kallikrein from serum α_2-globulin precursor and which causes a slow, sustained contraction of the smooth muscles.

Bursa of Fabricius: The hindgut organ located in the cloaca of birds that controls the ontogeny of B lymphocytes.

Bursal equivalent: The hypothetical organ or organs analogous to the bursa of Fabricius in nonavian species.

C region (constant region): The carboxy-terminal portion of the H or L chain that is identical in immunoglobulin molecules of a given class and subclass apart from genetic polymorphisms.

Capping: The movement of cell surface antigens toward one pole of a cell after the antigens are cross-linked by specific antibody.

Carcinoembryonic antigen (CEA): An antigen that is present on fetal endodermal tissue and is reexpressed on the surface of neoplastic cells, particularly in carcinoma of the colon.

Cardiolipin: A substance derived from beef heart, probably a component of mitochondrial membranes, that serves as an antigenic substrate for reagin or antitreponemal antibody.

Carrier: An immunogenic substance that, when coupled to a hapten, renders the hapten immunogenic.

Cationic proteins: Antimicrobial substances present within granules of phagocytic cells.

Cell-mediated immunity: Immunity in which the partici-

pation of lymphocytes and macrophages is predominant.

Cell-mediated lymphocytolysis: An in vitro assay for cellular immunity in which a standard mixed lymphocyte reaction is followed by destruction of target cells that are used to sensitize allogeneic cells during the MLC.

Centimorgan: A unit of physical distance on a chromosome, equivalent to a 1% frequency of recombination between closely linked genes. Also called a map unit.

Central lymphoid organs: Lymphoid organs that are essential to the development of the immune response, ie, the thymus and the bursa of Fabricius.

CH_{50} unit: The quantity or dilution of serum required to lyse 50% of the red blood cells in a standard hemolytic complement assay.

Chase-Sulzberger phenomenon: See **Sulzberger-Chase phenomenon.**

Chemiluminescence: Release of light energy by a chemical reaction usually involving reduction of an unstable intermediate to a stable one. Used as a means of measuring respiratory burst in phagocytic cells.

Chemokinesis: Reaction by which chemical substances determine rate of cellular movement.

Chemotaxis: A process whereby phagocytic cells are attracted to the vicinity of invading pathogens.

Chromatography: A variety of techniques useful for the separation of proteins.

Class I antigen: Histocompatibility antigen encoded in humans by A, B, and C loci and in mice by D and K loci.

Class II antigen: Histocompatibility antigen encoded in humans by DR, MB, MT, and Te loci and in mice by I and other loci.

Class III antigens: C4 and factor B are complement components encoded within the MHC.

Classic complement pathway: A series of enzyme-substrate and protein-protein interactions that ultimately leads to biologically active complement enzymes. It proceeds sequentially C1, 423 567 89.

Clonal anergy: Theory that B cell tolerance is induced by antigen-B cell contact during obligatory paralyzable phase or tolerance-sensitive phase of B cell differentiation.

Clonal deletion: A concept related to Burnet's clonal selection theory, which suggests that tolerance to self antigens results from deletion of autoreactive lymphocyte clones in embryonic life.

Clonal selection theory: The theory of antibody synthesis proposed by Burnet that predicts that the individual carries a complement of clones of lymphoid cells which are capable of reacting with all possible antigenic determinants. During fetal life, clones reacted against self antigens are eliminated on contact with antigen.

Clone: A group of cells all of which are the progeny of a single cell.

c-*myc* gene: Member of a candidate set of cancer-related genes or cellular oncogenes.

Coelomocyte: A wandering ameboid phagocyte found in all animal invertebrates containing a coelom.

Cohn fraction II: Primarily γ-globulin that is produced as the result of ethanol fractionation of serum according to the Cohn method.

Cold agglutinins: Antibodies that agglutinate bacteria or erythrocytes more efficiently at temperatures below 37 °C than at 37 °C.

Complement: A system of serum proteins that is the primary humoral mediator of antigen-antibody reactions.

Complement fixation: A standard serologic assay used for the detection of an antigen-antibody reaction in which complement is fixed as a result of the formation of an immune complex. The subsequent failure of lysis of sensitized red blood cells by complement that has been fixed indicates the degree of antigen-antibody reaction.

Complementarity: In genetics, the term indicates that more than one gene is required for the expression of a particular trait.

Concanavalin A (Con A): A lectin which is derived from the jack bean and which stimulates predominantly T lymphocytes.

Concentration catabolism effect: The direct effect exerted by the serum concentration of a plasma protein on its catabolic rate.

Concomitant immunity: The ability of a tumor-bearing animal to reject a test inoculum of its tumor at a site different from the primary site of tumor growth.

Congenic (originally **congenic resistant**)**:** Denotes a line of mice identical or nearly identical with other inbred strains except for the substitution at one histocompatibility locus of a foreign allele introduced by appropriate crosses with a second inbred strain.

Contact sensitivity: A type of delayed hypersensitivity reaction in which sensitivity to simple chemical compounds is manifested by skin reactivity.

Contrasuppression: Effects of immunoregulatory circuit that inhibit suppressor influences in a feedback loop.

Copolymer: A polymer of at least 2 different chemical moieties, eg, a polypeptide with 2 different amino acids.

Coproantibody: An antibody present in the lumen of the gastrointestinal tract.

Counterimmunoelectrophoresis: See **Electroimmunodiffusion.**

C-reactive protein (CRP): A β-globulin found in the serum of patients with diverse inflammatory diseases.

CREST phenomenon: A phenomenon which consists of *c*alcinosis, *R*aynaud's phenomenon, *e*sophageal dysmotility, *s*clerodactyly, and *t*elangiectasis and which occurs in patients with progressive systemic sclerosis.

Cross-reacting antigen: A type of tumor antigen present on all tumors induced by the same or a similar carcinogen.

Cross-reaction: The reaction of an antibody with an antigen other than the one that induced its formation.

Cryoglobulin: A protein that has the property of forming a precipitate or gel in the cold.

C terminus: The carboxy-terminal end of a protein molecule.

Cycle-specific drugs: Cytotoxic or immunosuppressive drugs that kill both mitotic and resting cells.

Cytokine: A factor such as a lymphokine or monokine produced by cells that affect other cells.

Cytotropic antibodies: Antibodies of the IgG and IgE classes that sensitize cells for subsequent anaphylaxis.

D gene region: Diversity region of genome encoding heavy chain sequences in the hypervariable region of immunoglobulin H chain.

Degranulation: A process whereby cytoplasmic granules of phagocytic cells fuse with phagosomes and dis-

charge their contents into the phagolysosome thus formed.

Delayed hypersensitivity: A cell-mediated immune reaction that can be elicited by subcutaneous injection of antigen, with a subsequent cellular infiltrate and edema that are maximal between 24 and 48 hours after antigen challenge.

Determinant groups: Individual chemical structures present on macromolecular antigens that determine antigenic specificity.

Dextrans: Polysaccharides composed of a single sugar.

Diapedesis: The outward passage of cells through intact vessel walls.

Direct agglutination: The agglutination of red cells, microorganisms, or other substances directly by serum antibody.

Direct immunofluorescence: The detection of antigens by fluorescently labeled antibody.

Distribution ratio: The fraction of total body protein located in plasma.

Disulfide bonds: Chemical S–S bonds between sulfhydryl-containing amino acids that bind together H and L chains as well as portions of H–H and L–L chains.

Domains (also **homology regions**): Segments of H or L chains that are folded 3-dimensionally and stabilized with disulfide bonds.

Dysgammaglobulinemia: A term not in common use that refers to a selective immunoglobulin deficiency.

E rosette: A formation of a cluster (rosette) of cells consisting of sheep erythrocytes and human T lymphocytes.

EAC rosette: A cluster of red cells (*e*rythrocytes) sensitized with *a*mboceptor (*a*ntibody) and *c*omplement around human B lymphocytes.

EAE (experimental allergic encephalomyelitis): An autoimmune disease in which an animal is immunized with homologous or heterologous extracts of whole brain, the basic protein of myelin, or certain polypeptide sequences within the basic protein, emulsified with Freund's complete adjuvant.

ECF-A (eosinophil chemotactic factor of anaphylaxis): An acidic peptide, molecular weight 500, that, when released, causes influx of eosinophils.

Effector cells: A term that usually denotes T cells capable of mediating cytotoxicity suppression or helper function.

Electroimmunodiffusion (counterimmunoelectrophoresis): An immunodiffusion technique in which antigen and antibody are driven toward each other in an electrical field and then precipitate.

Electrophoresis: The separation of molecules in an electrical field.

Encapsulation: A quasi-immunologic phenomenon in which foreign material is walled off within the tissues of invertebrates.

Endocytosis: The process whereby material external to a cell is internalized within a particular cell. It consists of pinocytosis and phagocytosis.

Endogenous pyrogen (IL-1): Factor produced by macrophages and other cells. Causes fever by reducing prostaglandins in region of hypothalamus.

Endotoxins: Lipopolysaccharides that are derived from the cell walls of gram-negative microorganisms and have toxic and pyrogenic effects when injected in vivo.

Enhancement: Improved survival of tumor cells in animals that have been previously immunized to the antigens of a given tumor.

Epitope: The simplest form of an antigenic determinant present on a complex antigenic molecule.

Equilibrium dialysis: A technique for measuring the strength or affinity with which antibody binds to antigen.

Equivalence: A ratio of antigen-antibody concentration where maximal precipitation occurs.

Euglobulin: A class of globulin proteins that are insoluble in water but soluble in salt solutions.

Exotoxins: Diffusible toxins produced by certain gram-positive and gram-negative microorganisms.

F_1 generation: The first generation of offspring after a designated mating.

F_2 generation: The second generation of offspring after a designated mating.

Fab: An antigen-binding fragment produced by enzymatic digestion of an IgG molecule with papain.

F(ab)'$_2$: A fragment obtained by pepsin digestion of immunoglobulin molecules containing the 2 H and 2 L chains linked by disulfide bonds. It contains antigen-binding activity. An F(ab)'$_2$ fragment and an Fc fragment comprise an entire monomeric immunoglobulin molecule.

Fc fragment: A crystallizable fragment obtained by papain digestion of IgG molecules that consists of the C-terminal half of 2 H chains linked by disulfide bonds. It contains no antigen-binding capability but determines important biologic characteristics of the intact molecule.

Fc receptor: A receptor present on various subclasses of lymphocytes for the Fc fragment of immunoglobulins.

Felton phenomenon: Immunologic unresponsiveness or tolerance induced in mice by the injection of large quantities of pneumococcal polysaccharide.

Fetal antigen: A type of tumor-associated antigen which is normally present on embryonic but not adult tissues and which is reexpressed during the neoplastic process.

Fibronectin: A protein that has an important role in the structuring of connective tissue.

Fluorescence: The emission of light of one color while a substance is irradiated with a light of a different color.

Forbidden clone theory: The theory proposed to explain autoimmunity that postulates that lymphocytes capable of self-sensitization and effector function are present in tolerant animals, since they were not eliminated during embryogenesis.

Fractional turnover rate: The percentage of plasma pool catabolized and cleared into the urine per day.

Francis skin test: An immediate hypersensitivity test for the presence of antibody on pneumococci in which pneumococcal capsular polysaccharide is injected into the skin and produces a wheal-and-flare response.

Freund's complete adjuvant: An oil-water emulsion that contains killed mycobacteria and enhances immune responses when mixed in an emulsion with antigen.

Freund's incomplete adjuvant: An emulsion that contains all of the elements of Freund's complete adjuvant with the exception of killed mycobacteria.

G cells: Gastrin-secreting cells in mucosa of the gastric antrum.

Gamma globulins: Serum proteins with gamma mobility in electrophoresis that comprise the majority of immunoglobulins and antibodies.

Gammopathy: A paraprotein disorder involving abnormalities of immunoglobulins.

Generalized anaphylaxis: A shocklike state that occurs within minutes following an appropriate antigen-antibody reaction resulting from the systemic release of vasoactive amines.

Genetic switch hypothesis: A hypothesis that postulates that there is a switch in the gene controlling heavy chain synthesis in plasma cells during the development of an immune response.

Genetic theory of antibody synthesis: A theory that predicts that information for synthesis of all types of antibody exists in the genome and that specific receptors are preformed on immunocompetent cells.

Germinal centers: A collection of metabolically active lymphoblasts, macrophages, and plasma cells that appears within the primary follicle of lymphoid tissues following antigenic stimulation.

Gm marker: An allotypic determinant on the heavy chain of human IgG.

Graft-versus-host (GVH) reaction: The clinical and pathologic sequelae of the reactions of immunocompetent cells in a graft against the cells of the histoincompatible and immunodeficient recipient.

Granulopoietin (colony-stimulating factor): A glycoprotein with a molecular weight of 45,000 derived from monocytes that controls the production of granulocytes by the bone marrow.

H chain: See **Heavy chain.**

H-2 locus: The major genetic histocompatibility region in the mouse.

Halogenation: A combination of a halogen molecule with a microbial cell wall that results in microbial damage.

Haplotype: That portion of the phenotype determined by closely linked genes of a single chromosome inherited from one parent.

Hapten: A substance that is not immunogenic but can react with an antibody of appropriate specificity.

Hassall's corpuscles (also **Leber's corpuscles** or **thymic corpuscles**): Whorls of thymic epithelial cells whose function is unknown.

HBcAg (also **core antigen**): The 27-nm core of hepatitis B virus which has been identified in the nuclei of hepatocytes.

HBeAg: Low-molecular-weight component of hepatitis B nucleocapsid indicating infectious state when present in serum.

HBsAg: The coat or envelope of hepatitis B virus.

Heavy chain (H chain): One pair of identical polypeptide chains making up an immunoglobulin molecule. The heavy chain contains approximately twice the number of amino acids and is twice the molecular weight of the light chain.

Heavy chain diseases: A heterogeneous group of paraprotein disorders characterized by the presence of monoclonal but incomplete heavy chains without light chains in serum or urine.

Helper T cells: A subtype of T lymphocytes that cooperate with B cells in antibody formation.

Hemagglutination inhibition: A technique for detecting small amounts of antigen in which the agglutination of antigen-coated red cells or other particles by specific antibody is inhibited by homologous antigen.

Hematopoietic system: All tissues responsible for production of the cellular elements of peripheral blood. This term usually excludes strictly lymphocytopoietic tissue such as lymph nodes.

Hemolysin: An antibody or other substance capable of lysing red blood cells.

Heterocytotropic antibody: An antibody that can passively sensitize tissues of species other than those in which the antibody is present.

Heterodimer: A 2-component molecule made up of different but closely joined segments, eg, T cell receptor α and β chains.

Heterologous antigen: An antigen that participates in a cross-reaction.

High dose (high zone) tolerance: Classic immunologic unresponsiveness produced by repeated injections of large amounts of antigen.

Hinge region: The area of the H chains in the C region between the first and second C region domains. It is the site of enzymatic cleavage into $F(ab)'_2$ and Fc fragments.

Histamine: A bioactive amine of MW 111 that causes smooth muscle contraction of human bronchioles and small blood vessels, increased permeability of capillaries, and increased secretion by nasal and bronchial mucous glands.

Histamine releasing factor: A lymphokine released from sensitized lymphocytes or antigenic stimulation that causes basophil histamine release.

Histocompatible: Sharing transplantation antigens.

HLA (human leukocyte antigen): The major histocompatibility genetic region in humans.

Homocytotropic antibody: An antibody that attaches to cells of animals of the same species.

Homologous antigen: An antigen that induces an antibody and reacts specifically with it.

Homopolymer: A molecule consisting of repeating units of a single amino acid.

Homozygous typing cells (HTC): Cells derived from an individual who is homozygous at the HLA-D locus used for MLR typing of the D locus in humans.

Horror autotoxicus: A concept introduced by Ehrlich, proposing that an individual is protected against autoimmunity or immunization against self antigens even though these antigens are immunogenic in other animals.

Hot antigen suicide: A technique in which an antigen is labeled with a high-specific-activity radioisotope (^{131}I). It is used either in vivo or in vitro to inhibit specific lymphocyte function by attachment to an antigen-binding lymphocyte, subsequently killing it by radiolysis.

Humoral: Pertaining to molecules in solution in a body fluid, particularly antibody and complement.

Hybridoma: Transformed cell line grown in vivo or in vitro that is a somatic hybrid of 2 parent cell lines and contains genetic material from both.

Hydrophilic: A hydrophilic compound is soluble in water; a hydrophilic group binds water on the exterior surfaces of proteins and cell membranes.

Hydrophobic: A hydrophobic compound is insoluble in water; a hydrophobic group is pushed to the interior of proteins or membranes, away from water.

Hyperacute rejection: An accelerated form of graft rejection that is associated with circulating antibody in the serum of the recipient and which can react with donor cells.

Hypervariable regions: At least 4 regions of extreme variability which occur throughout the V region of H and L chains and which determine the antibody combining site of an antibody molecule.

Hypogammaglobulinemia (agammaglobulinemia): A deficiency of all major classes of serum immunoglobulins.

I region: That portion of the major histocompatibility complex which contains genes that control immune responses.

Ia antigens (I region-associated antigens): Antigens that are controlled by Ir genes and are present on various tissues.

Idiotope: An epitope (antigenic determinant) on an idiotype.

Idiotype: A unique antigenic determinant present on homogeneous antibody or myeloma protein. The idiotype appears to represent the antigenicity of the antigen-binding site of an antibody and is therefore located in the V region.

IgA: The predominant immunoglobulin class present in secretions.

IgD: The predominant immunoglobulin class present on human B lymphocytes.

IgE: A reaginic antibody involved in immediate hypersensitivity reactions.

IgG: The predominant immunoglobulin class present in human serum.

IgM: A pentameric immunoglobulin comprising approximately 10% of normal human serum immunoglobulins, with a molecular weight of 900,000 and a sedimentation coefficient of 19S.

7S IgM: A monomeric IgM consisting of one monomer of 5 identical subunits.

I-J subregion: Part of mouse I region of the MHC that encodes for antigens present on suppressor cells and their active suppressor factors.

Immediate hypersensitivity: An unusual immunologic sensitivity to antigens that manifests itself by tissue reactions occurring within minutes after the antigen combines with its appropriate antibody.

Immune adherence: An agglutination reaction between a cell bearing C$\overline{423}$ and an indicator cell, usually a human red blood cell, which has a receptor for C3b.

Immune complexes: Antigen-antibody complexes.

Immune elimination: The enhanced clearance of an injected antigen from the circulation as a result of immunity to that antigen brought about by enhanced phagocytosis of the reticuloendothelial system.

Immune response genes (Ir genes): Genes that control immune responses to specific antigens.

Immune surveillance: A theory that holds that the immune system destroys tumor cells, which are constantly arising during the life of the individual.

Immunocytoadherence: A technique for identifying immunoglobulin-bearing cells by formation of rosettes consisting of these cells and red cells or other particles containing a homologous antigen.

Immunodominant: That part of an antigenic determinant which is dominant in binding with antibody.

Immunoelectrophoresis: A technique combining an initial electrophoretic separation of proteins followed by immunodiffusion with resultant precipitation arcs.

Immunofixation electrophoresis: Technique for identification of proteins by electrophoretic separation on a gel followed by precipitation in situ with specific antibodies.

Immunofluorescence: A histo- or cytochemical technique for the detection and localization of antigens in which specific antibody is conjugated with fluorescent compounds, resulting in a sensitive tracer that can be detected by fluorometric measurements.

Immunogen: A substance that, when introduced into an animal, stimulates the immune response. The term immunogen may also denote a substance that is capable of stimulating an immune response, in contrast to a substance that can only combine with antibody, ie, an antigen.

Immunogenicity: The property of a substance making it capable of inducing a detectable immune response.

Immunoglobulin: A glycoprotein composed of H and L chains that functions as antibody. All antibodies are immunoglobulins, but it is not certain that all immunoglobulins have antibody function.

Immunoglobulin class: A subdivision of immunoglobulin molecules based on unique antigenic determinants in the Fc region of the H chains. In humans there are 5 classes of immunoglobulins designated IgG, IgA, IgM, IgD, and IgE.

Immunoglobulin subclass: A subdivision of the classes of immunoglobulins based on structural and antigenic differences in the H chains. For human IgG there are 4 subclasses: IgG1, IgG2, IgG3, and IgG4.

Immunopotency: The capacity of a region of an antigen molecule to serve as an antigenic determinant and thereby induce the formation of specific antibody.

Immunoradiometry: A technique of radioimmunoassay that employs radiolabeled antibody rather than antigen.

Immunotherapy: Either hyposensitization in allergic diseases or treatment with immunostimulants or immunosuppressive drugs or biologic products.

Indirect agglutination (also **passive agglutination**): The agglutination of particles or red blood cells to which antigens have been coupled chemically.

Indirect immunofluorescence (also **double antibody immunofluorescence**): A technique whereby unlabeled antibody is incubated with substrate and then overlaid with fluorescently conjugated anti-immunoglobulin to form a sandwich.

Information theory of antibody synthesis: A theory that predicts that antigen dictates the specific structure of the antibody molecule.

Inoculation: The introduction of an antigen or antiserum into an animal in order to confer immunity.

Interferon: A heterogeneous group of low-molecular-weight proteins elaborated by infected host cells that protect noninfected cells from viral infection.

Interleukin-1: Macrophage-derived factor (previously called LAF, or leukocyte-activating factor) that promotes short-term proliferation of T cells.

Interleukin-2: Probable lymphocyte-derived factor (previously called TCGF, or T cell growth factor) that promotes long-term proliferation of T cell lines in culture.

Interleukin-3: A T cell product that induces proliferation and differentiation in other lymphocytes and some hematopoietic cells.

Inv marker: See **Km marker.**

Ir genes: See **Immune response genes.**

Isoagglutinin: An agglutinating antibody capable of agglutinating cells of other individuals of the same species in which it is found.

Isoantibody: An antibody that is capable of reacting with

an antigen derived from a member of the same species as that in which it is raised.

Isohemagglutinins: Antibodies to major red cell antigens present in members of a given species and directed against antigenic determinants on red cells from other members of the species.

Isotype: Antigenic characteristics of given class or subclass of immunoglobulin H and L chains.

J chain: A glycopeptide chain that is normally found in polymeric immunoglobulins, particularly IgA and IgM.

Jarisch-Herxheimer reaction: A local or occasionally generalized inflammatory reaction that occurs following treatment of syphilis and other intracellular infections; it is presumably caused by the release of large amounts of antigenic material into the circulation.

Jones criteria: Signs and symptoms used to diagnose acute rheumatic fever.

Jones-Mote reaction (cutaneous basophil hypersensitivity): A poorly understood type of delayed hypersensitivity with predominantly basophil infiltrate that occurs transiently 24 hours after cutaneous rechallenge with sensitizing antigen.

K cell: A killer cell responsible for antibody-dependent cell-mediated cytotoxicity.

K and D regions: Genetic loci in the major histocompatibility complex of the mouse, coding for H-2 antigens that are detectable serologically.

Kallikrein system: See **Kinin system.**

Kappa (κ) chains: One of 2 major types of L chains (qv).

Kinin: A peptide that increases vascular permeability and is formed by the action of esterases on kallikreins, which then act as vasodilators.

Kinin system (also **kallikrein system**): A humoral amplification system initiated by the activation of coagulation factor XII, eventually leading to the formation of kallikrein, which acts on an α-globulin substrate, kininogen, to form a bradykinin.

Km marker (also **Inv**): An allotypic marker on the κ L chain of human immunoglobulins.

Koch phenomenon: A delayed hypersensitivity reaction by tuberculin in the skin of a guinea pig following infection with *Mycobacterium tuberculosis*.

Kupffer cells: Fixed mononuclear phagocytes of the reticuloendothelial system that are present within the sinusoids of the liver.

Kveim test: A delayed hypersensitivity test for sarcoidosis in which potent antigenic extracts of sarcoid tissue are injected intradermally and biopsied 6 weeks later in order to observe the presence of a granuloma, indicating a positive test.

L chain: See **Light chain.**

Lactoferrin: An iron-containing compound that exerts a slight antimicrobial action by binding iron necessary for microbial growth.

Lambda (λ) chain: One of 2 major types of L chains (qv).

Langerhans cell: Bone marrow-derived macrophage with Ia cell surface antigens found in the epidermis.

Latex fixation test: An agglutination reaction in which latex particles are used to passively adsorb soluble protein and polysaccharide antigens.

LE cell phenomenon: Phagocytic leukocytes that have engulfed DNA, immunoglobulin, and complement and are present as a large homogeneous mass that is extruded from a damaged lymphocyte in systemic lupus erythematosus and other rheumatoid diseases.

Lectin: A substance that is derived from a plant and has panagglutinating activity for red blood cells. Lectins are commonly mitogens as well.

Leukocyte inhibitory factor (LIF): A lymphokine that inhibits the migration of polymorphonuclear leukocytes.

Leukocyte mitogenic factor (LMF): A lymphokine that will induce normal lymphocytes to undergo blast transformation and DNA synthesis.

Leukotriene: A vasodilatory lipoxygenase metabolite of arachidonic acid.

Levamisole: An anthelmintic drug with possible immunostimulatory capabilities.

Ligand: Any molecule that forms a complex with another molecule, such as an antigen used in a precipitin or radioimmunoassay.

Light chain (L chain): A polypeptide chain present in all immunoglobulin molecules. Two types exist in most species and are termed kappa (κ) and lambda (λ).

Linkage disequilibrium: An unexpected association of linked genes in a population.

Lipopolysaccharide (also **endotoxin**): A compound derived from a variety of gram-negative enteric bacteria that have various biologic functions including mitogenic activity for B lymphocytes.

Local anaphylaxis: An immediate hypersensitivity reaction that occurs in a specific target organ such as the gastrointestinal tract, nasal mucosa, or skin.

Locus: The specific site of a gene on a chromosome.

Low dose (low zone) tolerance: A transient and incomplete state of tolerance induced with small subimmunogenic doses of soluble antigen.

Lucio phenomenon (erythema necroticans): A variant of erythema nodosum leprosum in which necrotizing vasculitis produces crops of large polygonal lesions characterized by ulceration and sloughing of large areas of skin.

Lymphocyte: A mononuclear cell 7–12 μm in diameter containing a nucleus with densely packed chromatin and a small rim of cytoplasm.

Lymphocyte activation (also **lymphocyte stimulation, lymphocyte transformation,** or **blastogenesis**): An in vitro technique in which lymphocytes are stimulated to become metabolically active by antigen or mitogen.

Lymphocyte-defined (LD) antigens: A series of histocompatibility antigens that are present on the majority of mammalian cells and are detectable primarily by reactivity in the mixed lymphocyte reaction (MLR).

Lymphokines (also **mediators of cellular immunity**): Soluble products of lymphocytes that are responsible for the multiple effects of a cellular immune reaction.

Lymphotoxin (LT): A lymphokine that results in direct cytolysis following its release from stimulated lymphocytes.

Lysosomes: Granules that contain hydrolytic enzymes and are present in the cytoplasm of many cells.

Lysozyme (also **muramidase**): The cationic low-molecular-weight enzyme present in tears, saliva, and nasal secretions that reduces the local concentration of susceptible bacteria by attacking the mucopeptides of

their cell walls.

Lyt antigens: Differentiation antigens present on thymocytes and peripheral T cells.

M cells: Microfold cells that overlie lymphoid tissues in the small intestine and allow limited passage of intraintestinal antigens.

M protein: Antigenic component of surface of streptococci. Cross-reacts with muscle antigens.

M protein: See **Myeloma protein.**

Macrophage chemotactic factor (MCF): A lymphokine that selectively attracts monocytes or macrophages to the area of its release.

Macrophage-activating factor (MAF): A lymphokine that will activate macrophages to become avid phagocytic cells.

Macrophages: Phagocytic mononuclear cells that derive from bone marrow monocytes and subserve accessory roles in cellular immunity.

Major histocompatibility complex (MHC): An as yet undetermined number of genes located in close proximity that determine histocompatibility antigens of members of a species.

Mast cell: A tissue cell that resembles a peripheral blood basophil and contains granules with serotonin and histamine present.

β_2-Microglobulin: A protein (MW 11,600) which is associated with the outer membrane of many cells, including lymphocytes, and which may function as a structural part of the histocompatibility antigens on cells.

Migration inhibitory factor (MIF): A lymphokine that is capable of inhibiting the migration of macrophages.

Mithridatism (after Mithridates, king of Pontus): Immunity induced against poisons by the administration of gradually increasing doses of the poison.

Mitogens (also **phytomitogens**): Substances that cause DNA synthesis, blast transformation, and ultimately division of lymphocytes.

Mixed lymphocyte culture (mixed leukocyte culture) (MLC): An in vitro test for cellular immunity in which lymphocytes or leukocytes from genetically dissimilar individuals are mixed and mutually stimulate DNA synthesis.

Mixed lymphocyte reaction (MLR): See **Mixed lymphocyte culture.**

Monoclonal hypergammaglobulinemia: An increase in immunoglobulins produced by a single clone of cells containing one H chain class and one L chain type.

Monoclonal immunoglobulin molecules: Identical copies of antibody that consist of one H chain class and one L chain type.

Monoclonal protein: A protein produced from the progeny of a single cell called a clone.

Monokine: Substance released from macrophage or monocyte that affects the function of another cell.

Monomer: The basic unit of an immunoglobulin molecule that is comprised of 4 polypeptide chains: 2 H and 2 L.

Multiple myeloma: A paraproteinemic disorder consisting typically of the presence of serum paraprotein, anemia, and lytic bone lesions.

Myeloma protein (M protein): Either an intact monoclonal immunoglobulin molecule or a portion of one produced by malignant plasma cells.

Myeloperoxidase: An enzyme that is present within granules of phagocytic cells and catalyzes peroxidation of a variety of microorganisms.

Natural antibody: Antibody present in the serum in the absence of apparent specific antigenic contact.

NBT test: A metabolic assay involving the reduction of nitroblue tetrazolium dye during activation of the hexose monophosphate shunt in phagocytic cells.

Neoantigens: Nonself antigens that arise spontaneously on cell surfaces, usually during neoplasia.

Nephelometry: The measurement of turbidity or cloudiness in a suspension or a solution.

Nephritic factor: Serum immunoglobulin with conglutinin activity that can activate the alternative complement pathway. Often present in serum of patients with membranoproliferative glomerulonephritis.

Network hypothesis: Jerne's theory of immunoregulation by a cascade of idiotype-anti-idiotype reactions involving T cell receptors and antibodies.

Neutralization: The process by which antibody or antibody in complement neutralizes the infectivity of microorganisms, particularly viruses.

Neutrophil microbicidal assay: A test for the ability of neutrophils to kill intracellular bacteria.

NK cells (natural killer cells): Cytotoxic cells belonging to the cell class responsible for cellular cytotoxicity without prior sensitization.

Nonresponder: An animal unable to respond to an antigen, usually because of genetic factors.

N terminus: The amino-terminal end of a protein molecule.

Nucleoside phosphorylase: An enzyme that catalyzes the conversion of inosine to hypoxanthine and is rarely deficient in patients with immunodeficiency disorders.

Nude mouse: A hairless mouse that congenitally lacks a thymus and has a marked deficiency of thymus-derived lymphocytes.

Null cells: Cells lacking the specific identifying surface markers for either T or B lymphocytes.

NZB mouse: A genetically inbred strain of mice in which autoimmune disease resembling systemic lupus erythematosus develops spontaneously.

Oligoclonal bands: Immunoglobulins with restricted electrophoretic mobility in agarose gels found in cerebrospinal fluid of patients with multiple sclerosis and some other central nervous system diseases.

Oncogene: A gene of either viral or mammalian origin that causes transformation of cells in culture.

Oncogenesis: The process of producing neoplasia or malignancy.

Ontogeny: The developmental history of an individual organism within a group of animals.

Opsonin: A substance capable of enhancing phagocytosis. Antibodies and complement are the 2 main opsonins.

Osteoclast activating factor (OAF): A lymphokine that promotes the resorption of bone.

Ouchterlony double diffusion: An immunoprecipitation technique in which antigen and antibody are allowed to diffuse toward each other and form immune complexes in agar.

Palindrome: In molecular biology, a self-complementary length of DNA which when read from the 5′ to the 3′ end displays an equivalent sequence whether read from the left or the right or forward or backward.

Paralysis: The pseudotolerant condition in which an ongoing immune response is masked by the presence of overwhelming amounts of antigen.

Paraproteinemia: A condition occurring in a heterogeneous group of diseases characterized by the presence in serum or urine of a monoclonal immunoglobulin.

Paratope: An antibody combining site for epitope, the simplest form of an antigenic determinant.

Passive cutaneous anaphylaxis (PCA): An in vivo passive transfer test for recognizing cytotropic antibody responsible for immediate hypersensitivity reactions.

Passive immunity: Protection achieved by introduction of preformed antibody or immune cells into a nonimmune host.

Patching: The reorganization of a cell surface membrane component into discrete patches over the entire cell surface.

Peripheral lymphoid organs: Lymphoid organs not essential to the ontogeny of immune responses, ie, the spleen, lymph nodes, tonsils, and Peyer's patches.

Peritoneal exudate cells (PEC): Inflammatory cells present in the peritoneum of animals injected with an inflammatory agent.

Peyer's patches: Collections of lymphoid tissue in the submucosa of the small intestine that contain lymphocytes, plasma cells, germinal centers, and T cell-dependent areas.

Pfeiffer phenomenon: A demonstration showing that cholera vibrios introduced into the peritoneal cavity of an immune guinea pig lose their mobility and are lysed regardless of the presence of cells.

Phagocytes: Cells that are capable of ingesting particulate matter.

Phagocytosis: The engulfment of microorganisms or other particles by leukocytes.

Phagolysosome: A cellular organelle that is the product of the fusion of a phagosome and a lysosome.

Phagosome: A phagocytic vesicle bounded by inverted plasma membrane.

Phylogeny: The developmental and evolutionary history of a group of animals.

Phytohemagglutinin (PHA): A lectin which is derived from the red kidney bean *(Phaseolus vulgaris)* and which stimulates predominantly T lymphocytes.

Phytomitogens: Glycoproteins that are derived from plants and stimulate DNA synthesis and blast transformation in lymphocytes.

Pinocytosis: The ingestion of soluble materials by cells.

Plaque-forming cells: Antibody-producing cells capable of forming a hemolytic plaque in the presence of complement and antigenic erythrocytes.

Plasma cells: Fully differentiated antibody-synthesizing cells that are derived from B lymphocytes.

Plasma half-life (half time, $t_{1/2}$): The time necessary for 50% of a passively infused protein to disappear from serum.

Plasmin: A fibrinolytic enzyme capable of proteolytically digesting C1.

Plasminogen activator: An enzyme secreted by macrophages that converts a plasma zymogen to active plasmin.

Pokeweed mitogen (PWM): A lectin which is derived from pokeweed *(Phytolacca americana)* and which stimulates both B and T lymphocytes.

Polyclonal hypergammaglobulinemia: An increase in γ-globulin of various classes containing different H and L chains.

Polyclonal mitogens: Mitogens that activate large subpopulations of lymphocytes.

Polyclonal proteins: A group of molecules derived from multiple clones of cells.

Polymers: Immunoglobulins composed of more than a single basic monomeric unit, eg, an IgA dimer consists of 2 units.

Postcapillary venules: Specialized blood vessels lined with cuboid epithelium located in the paracortical region of lymph nodes through which lymphocytes traverse.

Pre-B cells: Large immature lymphoid cells with diffuse cytoplasmic IgM that eventually develop into B cells.

Precipitation: A reaction between a soluble antigen and soluble antibody in which a complex lattice of interlocking aggregates forms.

Primary follicles: Tightly packed aggregates of lymphocytes found in the cortex of the lymph node or in the white pulp of the spleen after antigenic stimulation. Primary follicles develop into germinal centers.

Primed lymphocyte typing (PLT): A variation on the MLR in which cells are primed by allogeneic stimulation and reexposed to fresh stimulator cells. Used to type for HLA-D determinants.

Private antigen: A tumor or histocompatibility antigen restricted either to a specific chemically induced tumor or to the specific product of a given allele.

Procoagulant factor (tissue factor): Lymphokine that can replace factor VIII activity in factor VIII-deficient plasma.

Properdin system: A group of proteins involved in resistance to infection. The 2 main constitutents consist of factor A and factor B. Properdin factor A is identical with C3, a β-globulin of MW 180,000. Properdin factor B is a β_2-globulin of MW 95,000. It is also called C3 proactivator, glycine-rich β-glycoprotein (GBG), or β_2-glycoprotein II. Properdin factor D is an α-globulin of MW 25,000 also called C3 proactivator convertase or glycine-rich β-glycoproteinase (GBGase).

Prostaglandins: A variety of naturally occurring aliphatic acids with various biologic activities, including increased vascular permeability, smooth muscle contraction, bronchial constriction, and alteration in the pain threshold.

Prothymocytes: Immature precursors of mature thymocytes that develop within the thymus gland.

Prozone phenomenon: Suboptimal precipitation that occurs in the region of antibody excess during immunoprecipitation reactions.

Public antigen: Determinant common to several distinct or private antigens.

Pyogenic microorganisms: Microorganisms whose presence in tissues stimulates an outpouring of polymorphonuclear leukocytes.

Pyrogens: Substances which are released either endogenously from leukocytes or administered exogenously, usually from bacteria, and which produce fever in susceptible hosts.

Pyroglobulins: Monoclonal immunoglobulins that precipitate irreversibly when heated to 56 °C.

Qa locus: Genetic locus mapping between H2-D and TL in mice which encodes Qa antigen found on both Ts and Th cells.

Quellung: The swelling of the capsules of pneumococci when the organisms are exposed to pneumococcal antibodies.

Radioallergosorbent test (RAST): A radioimmunoassay capable of detecting IgE antibody directed at specific allergens.

Radioimmunoassay: A variety of immunologic techniques in which a radioactive isotope is used to detect antigens or antibodies in some form of immunoassay.

Radioimmunodiffusion (Rowe's method): A modification of immunodiffusion in which a radioactive antibody is incorporated in order to increase the sensitivity by means of autoradiography.

Radioimmunosorbent test (RIST): A solid phase radioimmunoassay that can detect approximately 1 ng of IgE.

Ragocytes (RA cells): Polymorphonuclear leukocytes that have ingested characteristic dense IgG aggregates, rheumatoid factor, complement, and fibrin. They are found in the joints of patients with rheumatoid arthritis.

Raji cell test: An assay for immune complexes using the Raji lymphoblastoid cell line.

Reagin: Synonymous with IgE antibody. Also denotes a complement-fixing antibody that reacts in the Wassermann reaction with cardiolipin.

Recombinant: An animal that has experienced a recombinational event during meiosis, consisting of crossover and recombination of parts of 2 chromosomes.

Recombinatorial germ line theory: Theory proposed by Dreyer and Bennett which states that variable region and constant region immunoglobulin genes are separated and rejoined at DNA levels.

Rejection response: An immune response with both humoral and cellular components directed against transplanted tissue.

Reticuloendothelial system: A mononuclear phagocytic system located primarily in the reticular connective tissue framework of the spleen, liver, and lymphoid tissues.

Rheumatoid factor (RF): An anti-immunoglobulin antibody directed against denatured IgG present in the serum of patients with rheumatoid arthritis and other rheumatoid diseases.

Ricin: A poisonous substance that derives from the seed of the castor oil plant and agglutinates red blood cells (a lectin).

Rocket electrophoresis (Laurell technique): An electro-immunodiffusion technique in which antigen is electrophoresed into agar containing specific antibody and precipitates in a tapered rocket-shaped pattern. This technique is used for quantitation of antigens.

Rose-Waaler test: A type of passive hemagglutination test for the detection of rheumatoid factor that employs tanned red blood cells coated with rabbit 7S IgG antibodies specific for sheep red blood cells.

S region: The chromosomal region in the H-2 complex containing the gene for a serum β-globulin.

S value: Svedberg unit. Denotes the sedimentation coefficient of a protein, determined usually by analytic ultracentrifugation.

Schultz-Dale test: An in vitro assay for immediate hypersensitivity in which smooth muscle is passively sensitized by cytotropic antibody and contracts after the addition of an antigen.

Second set graft rejection: An immunologic rejection of a graft in a host that is immune to antigens contained in that graft.

Secretory IgA: A dimer of IgA molecules with a sedimentation coefficient of 11S, linked by J chain and secretory component.

Secretory immune system: A distinct immune system that is common to external secretions and consists predominantly of IgA.

Secretory piece (T piece): A molecule of MW 70,000 produced in epithelial cells and associated with secretory immunoglobulins, particularly IgA and IgM.

Sensitized: Synonymous with immunized.

Sensitizer: A term introduced by Pfeiffer to denote a specific thermostable factor capable of bacterial lysis when combined with alexin.

Sequential determinants: Determinants whose specificity is dictated by the sequence of subunits within the determinant rather than by the molecular structure of the antigen molecule.

Serologically defined (SD) antigens: Antigens that are present on membranes of nearly all mammalian cells and are controlled by genes present in the major histocompatibility complex. They can be easily detected with antibodies.

Serology: Literally, the study of serum. Refers to the determination of antibodies to infectious agents important in clinical medicine.

Serotonin (5-hydroxytryptamine): A catecholamine of MW 176 that is stored in murine mast cells and human platelets and has a pharmacologic role in anaphylaxis in most species except humans.

Serum sickness: An adverse immunologic response to a foreign antigen, usually a heterologous protein.

Shwartzman phenomenon: A nonimmunologic phenomenon that results in tissue damage both at the site of injection and at widespread sites following the second of 2 injections of endotoxin.

Side chain theory: A theory of antibody synthesis proposed by Ehrlich in 1896 suggesting that specific side chains that form antigen receptors are present on the surface membranes of antibody-producing cells.

Single radial diffusion (radioimmunodiffusion): A technique for quantitating antigens by immunodiffusion in which antigen is allowed to diffuse radially into agar containing antibody. The resultant precipitation ring reflects the concentration of the antigen.

Skin-reactive factor (SRF): A lymphokine that is responsible for vasodilatation and increased vascular permeability.

Slow virus: A virus that produces disease with a greatly delayed onset and protracted course.

Solid phase radioimmunoassay: A modification of radioimmunoassay in which antibody is adsorbed onto solid particles or tubes.

Spermine: A polyamine present in prostatic secretions that is a pH-dependent inhibitor of gram-positive microorganisms.

Spherulin: A spherule-derived antigen from *Coccidioides immitis* used in delayed hypersensitivity skin testing for coccidioidomycosis.

SRS-A: An acidic lipoprotein, MW about 400, that has a prolonged constrictive effect on smooth muscle.

SS-A: Antibody to RNA found in Sjögren's syndrome and also associated with heart block in infants born to mothers with this antibody.

SS-B: Antibody to RNA found in Sjögren's syndrome and other rheumatic diseases.

Sulzberger-Chase phenomenon: Abrogation of dermal contact sensitivity to various chemicals produced by prior oral feeding of the specific agent.

Suppressor T cells: A subset of T lymphocytes that suppress antibody synthesis by B cells or inhibit other cellular immune reactions by effector T cells.

Surface phagocytosis: The enhancement of phagocytosis by entrapment of organisms on surfaces such as leukocytes, fibrin clots, or other tissue surfaces.

Switch: Refers to change in synthesis between heavy chains within a single immunocyte from μ to γ—eg, during differentiation. V regions are not affected by H chain switch.

Syngeneic: Denotes the relationship that exists between genetically identical members of the same species.

T antigens: Tumor antigens, probably protein products of the viral genome present only on infected neoplastic cells.

T cell (T lymphocyte): A thymus-derived cell that participates in a variety of cell-mediated immune reactions.

T cell rosette: See **E rosette.**

T piece: See **Secretory piece.**

Thelolymphocytes: Small lymphocytes that are found in contiguity with intestinal epithelial cells and whose function is unknown.

Theta antigen: An alloantigen present on the surface of most thymocytes and peripheral T lymphocytes.

Thymopoietin (originally **thymin**): A protein of MW 7000 which is derived originally from the thymus of animals with autoimmune thymitis and myasthenia gravis and which can impair neuromuscular transmission.

Thymosin: A thymic hormone protein of MW 12,000 that can restore T cell immunity in thymectomized animals.

Thymus: The central lymphoid organ that is present in the thorax and controls the ontogeny of T lymphocytes.

Thymus-dependent antigen: Antigen that depends on T cell interaction with B cells for antibody synthesis, eg, erythrocytes, serum proteins, and hapten-carrier complexes.

Thymus-independent antigen: Antigen that can induce an immune response without the apparent participation of T lymphocytes.

Tissue factor: See **Procoagulant factor.**

TL antigen: A membrane antigen which is present on prothymocytes in mice with a TL+ gene, but which is lost during thymic maturation.

Tolerance: Traditionally denotes that condition in which responsive cell clones have been eliminated or inactivated by prior contact with antigen, with the result that no immune response occurs on administration of antigen.

Toxoids: Antigenic but nontoxic derivatives of toxins.

Transcription: The synthesis of RNA molecules from a DNA template.

Transfer factor: A dialyzable extract of immune lymphocytes that is capable of transferring cell-mediated immunity in humans and possibly in other animal species.

Translation: The process of formation of a peptide chain from individual amino acids to form a protein molecule.

Transplantation antigens: Those antigens which are expressed on the surface of virtually all cells and which induce rejection of tissues transplanted from one individual to a genetically disparate individual.

Trophoblast: Cell layer in placenta in contact with uterine lining. Produces various immunosuppressive substances, eg, hormones.

Tryptic peptides: Peptides produced as a result of tryptic digestion of a protein molecule.

Tuftsin: A 4-amino-acid (threonine-lysine-proline-arginine) polypeptide that enhances macrophage functions.

Tumor-associated antigens (TAA): Cell surface antigens that are expressed on malignant but not normal cells.

Ultracentrifugation: A high-speed centrifugation technique that can be used for the analytic identification of proteins of various sedimentation coefficients or as a preparative technique for separating proteins of different shapes and densities.

Ultrafiltration: The filtration of solutions or suspensions through membranes of extremely small graded pore sizes.

V antigens: Virally induced antigens that are expressed on viruses and virus-infected cells.

V (variable) region: The amino-terminal portion of the H or L chain of an immunoglobulin molecule, containing considerable heterogeneity in the amino acid residues compared to the constant region.

V region subgroups: Subdivisions of V regions of kappa chains based on substantial homology in sequences of amino acids.

Vaccination: Immunization with antigens administered for the prevention of infectious diseases (term originally coined to denote immunization against vaccinia or cowpox virus).

Variolation: Inoculation with a virus of unmodified smallpox (variola).

Viscosity: The physical property of serum that is determined by the size, shape, and deformability of serum molecules. The hydrostatic state, molecular charge, and temperature sensitivity of proteins.

Von Krogh equation: An equation that relates complement to the degree of lysis of red blood cells coated with anti-red blood cell antibodies under standard conditions. Used to determine hemolytic complement titers in serum.

Wasting disease (also **runt disease**): A chronic, ultimately fatal illness associated with lymphoid atrophy in mice who are neonatally thymectomized.

Xenogeneic: Denotes the relationship that exists between members of genetically different species.

Xenograft: A tissue or organ graft between members of 2 distinct or different species.

Zone electrophoresis: Electrophoresis performed on paper or cellulose acetate in which proteins are separated almost exclusively on the basis of charge.

Acronyms & Abbreviations Commonly Used in Immunology

ABA Azobenzenearsenate.
ABPA Allergic bronchopulmonary aspergillosis.
ACTH Adrenocorticotropic hormone.
ADA Adenosine deaminase.
ADCC Antibody-dependent cell-mediated cytotoxicity.
AEF Allogeneic effect.
AFC Antibody-forming cells.
AFP Alpha-fetoprotein.
AGN Acute glomerulonephritis.
AHA Autoimmune hemolytic anemia.
AHG Antihemophilic globulin.
AIDS Acquired immunodeficiency syndrome.
AIHA Autoimmune hemolytic anemia.
ALG Antilymphocyte globulin.
ALS Antilymphocyte serum.
Am Allotypic marker on IgA.
AMA Antimitochondrial antibodies.
AMP Adenosine monophosphate.
ANA Antinuclear antibody.
ANF Antinuclear factor.
APC Antigen-presenting cells.
APSGN Acute poststreptococcal glomerulonephritis.
ARC AIDS-related complex.
ASO Antistreptolysin O
ATG Antithymocyte globulin.

B27 HLA antigen with strong disease association.
BAF B cell-activating factor.
BAL Dimercaprol (British anti-Lewisite).
Balb/c Inbred strain of mice.
BCG Bacillus Calmette-Guérin.
BCDF B cell differentiation factors.
BCGF B cell growth factors.
Bf Properdin factor B.
BFP Biologic false-positive (tests for syphilis).
BJ Bence Jones.
BPI Bactericidal permeability-increasing protein.
BPO Benzyl penicilloyl.
BSA Bovine serum albumin.
BUDR, BUdR 5-Bromodeoxyuridine.

CAH Chronic active hepatitis.
CALLA Common acute lymphocytic leukemia antigen.
cAMP Cyclic adenosine monophosphate.
CCF Crystal-induced chemotactic factor.
CD Cluster of differentiation.
CD4 An antigenic marker of helper/inducer T cells (also designated OKT 4, T4, Leu 3).
CD8 An antigenic marker of suppressor/cytotoxic T cells (also designated OKT 8, T8, Leu 2).
CEA Carcinoembryonic antigen.
CF Complement fixation.
CFA Colonization factor antigens (also Freund's complete adjuvant).
CFU Colony-forming unit.
CFU-C Colony-forming unit of cells grown in culture.
CFU-S Colony-forming unit of cells grown in the spleen.
CGD Chronic granulomatous disease.
cGMP Cyclic guanosine monophosphate.
C_H Constant domain of H chain.
CHS Chédiak-Higashi syndrome.
C_L Constant domain of L chain.
cM Centimorgan.
c-myc An oncogene.
CMC Chronic mucocutaneous candidiasis.
CMI Cell-mediated immunity.
CML Cell-mediated lympholysis.
CMPGN Chronic membranoproliferative glomerulonephritis.
CMV Cytomegalovirus(es).
C3NeF C3 nephritic factor.
Con A Concanavalin A.
C3PA C3 proactivator.
CPGN Chronic proliferative glomerulonephritis.
CR1–CR6 Six distinct receptors for C3 fragments found on various cell types.
CRP C-reactive protein.
CSA Colony-stimulating activity.
CSF Colony-stimulating factor.
CTL Cytotoxic lymphocytes.
CTLL Cloned mouse cytotoxic T lymphocytic line.

DDS Dapsone (diaminodiphenylsulfone).
DEAE Diethylaminoethyl.
DGI Disseminated gonococcal infection.
D_H Diversity region of immunoglobulin heavy-chain gene.
DIC Disseminated intravascular coagulation.
D-L Donath-Landsteiner.
DLE Dialyzable leukocyte extracts; disseminated lupus erythematosus.
DNCB 2,4-Dinitrochlorobenzene.
DNFB Dinitrofluorobenzene.
DNP Dinitrophenyl.
DP Human class II MHC allele (formerly called SB).
DPO Dimethoxyphenylpenicilloyl.
DQ Human class II MHC allele (formerly called DC, MB, and DS).
DR D-related HLA locus in humans.
DSCG Disodium cromoglycate.
DST Donor-specific transfusion.
DT Diphtheria and tetanus toxoids.
DTH Delayed-type hypersensitivity.
DTP Diphtheria and tetanus toxoid combined with pertussis vaccine.

EA Early antigens (of EBV).
EA Erythrocyte amboceptor (sensitized erythrocytes).
EAC Erythrocyte amboceptor complement.
EAE Experimental allergic encephalitis or encephalomyelitis.
EAN Experimental allergic neuritis.
EB Epstein-Barr.
EBNA Epstein-Barr virus nuclear antigen.
EBV Epstein-Barr virus.
ECF Eosinophil chemotactic factor.

ECF-A Eosinophil chemotactic factor of anaphylaxis.
ECP Eosinophil cationic protein.
EDTA Ethylenediaminetetraacetate.
EFA Enhancing factor of allergy.
EIA Enzyme immunoassay.
ELISA Enzyme-linked immunosorbent assay.
EMIT Enzyme multiple immunoassay technique; a homogeneous enzyme immunoassay.
ENA Extractable nuclear antigen.
EP Endogenous pyrogen.
EPO Eosinophilic peroxidase.
ER Endoplasmic reticulum.
ESR Erythrocyte sedimentation rate.

F_1 First generation.
F_2 Second generation.
FA Fluorescent antibody.
FAB French, American, British (system of leukemia classification).
Fab Antigen-binding fragment.
FACS Fluorescent-activated cell sorter.
Fc Crystallizable fragment.
FCR Fractional catabolic rate.
FcRγ Fc receptor specific for IgG.
FcRμ Fc receptor specific for IgM.
FeLV Feline leukemia virus.
FEV Forced expiratory volume in 1 second.
FITC Fluorescein isothiocyanate.
FSH Follicle-stimulating hormone.
FTA-ABS Fluorescent treponemal antibody absorption test.
FUDR, FUAR Fluorodeoxyuridine.

GALT Gut-associated lymphoid tissue.
GBG Glycine-rich beta-glycoprotein.
GBM Glomerular basement membrane.
GEF Glycosylation-enhancing factor.
GFR Glomerular filtration rate.
GGG Glycine-rich gamma-glycoprotein.
GIF Glycosylation inhibition factor.
GLO Glyoxylase.
Gm Allotypic marker on human IgG.
GMP Guanosine monophosphate.
gp70 Glycoprotein antigen (MW 70,000) on viral envelope of C type murine viruses.
GPA Guinea pig albumin.
GPC Gastric parietal cell.
G6PD Glucose-6-phosphate dehydrogenase.
GVH Graft-versus-host (disease).
GVHR Graft-versus-host reaction.

HAA Hepatitis-associated antigen.
HAE, HANE Hereditary angioneurotic edema.
HAT Hypoxanthine, aminopterin, and thymidine.
HAV Hepatitis A virus.
HbA Adult hemoglobin.
HBcAg Low-molecular-weight nucleocapsid antigen of hepatitis B virus.
HbF Fetal hemoglobin.
HBsAg Hepatitis B surface antigen.
HBV Hepatitis B virus.
HCD Heavy chain disease.
hCG Human chorionic gonadotropin.
HDL High-density lipoproteins.
HDN Hemolytic disease of newborn.
H&E Hematoxylin and eosin (stain).
HETE Hydroxyeicosatetraenoic acid.
HEV High endothelial venules.
HI Hemagglutination inhibition.
HIV Human immunodeficiency virus.
HLA Human leukocyte antigen.
$(H_2L_2)n$ General formula for immunoglobulin molecule.
HMP Hexose monophosphate (shunt).
HMW-NCF High-molecular-weight neutrophil chemotactic factor.
HPETE Hydroperoxyeicosatetraenoic acid.
HPRT Hypoxanthine phosphoribosyl transferase.
HSA Human serum albumin.
HSF Histamine-sensitizing factor.
HSV Herpes simplex virus.
5-HT 5-Hydroxytryptamine (serotonin).
HTC Homozygous typing cells.
HTLV Human T cell leukemia virus.

ICA Islet cell antibody.
ICSA Islet cell surface antibody.
IDDM Insulin-dependent diabetes mellitus.
IDU Idoxuridine.
IEF Isoelectric focusing.
IEP Immunoelectrophoresis.
IF Intrinsic factor (also initiating factor).
IFA Indirect fluorescent antibody.
IFN Interferon.
IL-1 Interleukin-1.
IL-2 Interleukin-2.
IL-3 Interleukin-3.
INH Isoniazid (isonicotinic acid hydrazide).
Inv Allotypic marker on human kappa chain (κm).
Ir Immune response (genes).
I-RNA Immune RNA.
ISG Immune serum globulin.
ITP Idiopathic thrombocytopenic purpura.

J segment Joining segment of DNA encoding immunoglobulins.
JH Joining region of immune globulin-bearing chains.
JRA Juvenile rheumatoid arthritis.

K (cells) Killer (cells).
KAF Bovine conglutinin: conglutinin activating factor.
KLH Keyhole limpet hemocyanin.

La See SS-B.
LAF Leukocyte-activating factor (see IL-1).
LAK Lymphokine-activated killer (cells).
LAV Lymphadenopathy-associated virus.
LCMV Lymphocytic choriomeningitis virus.
LD Lymphocyte-defined.
LDCC Lectin-dependent cell-mediated cytotoxicity.
LDCF Lymphocyte-derived chemotactic factors.
LDH Lactate dehydrogenase.
LDL Low-density lipoproteins.
LE Lupus erythematosus.
Lf Limit flocculation (unit) (1/1000 Lf = 0.0000003 mg).
LGL Large granular lymphocytes.
LH Luteinizing hormone.
LIF Leukocyte inhibitory factor.
LMI Leukocyte migration inhibition.
LPS Lipopolysaccharide.
LT Leukotriene.
LT Lymphotoxin or lymphocytotoxin.
L3T4 Mouse homolog of CD4; a helper T cell antigen in humans.
Lyb Lymphocyte antigens on murine B cells.
LyNeF Lytic nephritic factor.
Lyt Lymphocyte antigens on murine T cells.

MAC (Complement) membrane attack complex.
MAF Macrophage-activating (-arming) factor.

MALT Mucosa-associated lymphoid tissue.
MBP Major basic protein.
MC(DC) Human MHC antigen of class II type.
MCA Methylcholanthrene.
MCF Macrophage chemotactic factor.
MCGN Mesangiocapillary (membranoproliferative) glomerulonephritis.
MCTD Mixed connective tissue disease.
MDP Muramyl dipeptide.
MeBSA Methylated bovine serum albumin.
MER Methanol extraction residue (of phenol-treated BCG).
MF Mitogenic factor.
MHA Major histocompatibility antigen.
MHC Major histocompatibility complex.
MHD Minimum hemolytic dilution or dose.
MIF Migration inhibitory factor.
MLC Mixed lymphocyte (leukocyte) culture.
MLD Minimum lethal dose.
MLR Mixed lymphocyte (or leukocyte) response or reaction.
MMI Macrophage migration inhibition.
6-MP Mercaptopurine.
MPG Methyl green pyronin.
MPO Myeloperoxidase.
MS Multiple sclerosis.
MTX Methotrexate.
MuLV Murine leukemia virus.
MW Molecular weight.

NBT Nitroblue tetrazolium.
NF Nephritic factor.
NK Natural killer (cells).
NZB New Zealand black (mice).
NZW New Zealand white (mice or rabbits).

OAF Osteoclast activating factor.
OPV Oral poliovirus.
OS Obese strain.
OT Old tuberculin.

PA Pernicious anemia.
PAF Platelet-activating factor.
PAIDS AIDS in pediatric patients.
PAP Peroxidase antiperoxidase.
PAS *p*-Aminosalicylic acid; periodic acid-Schiff (reaction).
PBC Primary biliary cirrhosis.
PCA Passive cutaneous anaphylaxis.
PCM Protein-calorie malnutrition.
PEC Peritoneal exudate cells.
PF/dil Permeability factor dilute.
PFC Plaque-forming cells.
PG Prostaglandin.
Pg5 Urinary pepsinogen.
PGE Prostaglandin E (PGE_1, PGE_2, $PGE_{2\alpha}$).
PGM_3 Phosphoglucomutase 3.
PGN Proliferative glomerulonephritis.
PHA Phytohemagglutinin.
PIE Pulmonary infiltration with eosinophilia.
pIgA Polymeric IgA.
PK (P-K) Prausnitz-Küstner (reaction).
PLL Poly-L-lysine.
PLT Primed lymphocyte typing.
PMA Phorbol myristate acetate; a tumor promotor that stimulates monocytes and lymphocytes nonspecifically.
PML Progressive multifocal leukodystrophy.
PMN Polymorphonuclear neutrophil.
PNA Peanut agglutinin.
PNH Paroxysmal nocturnal hemoglobulinuria.
PPD Purified protein derivative (tuberculin).
PSS Progressive systemic sclerosis.
PVP Polyvinylpyrrolidone.
PWM Pokeweed mitogen.

Qa Region on 17th mouse chromosome in the MHC that encodes a class of T cell antigens.

RA Rheumatoid arthritis.
Ragg Rheumatoid agglutinin.
RANA Rheumatoid arthritis nuclear antigen.
RAST Radioallergosorbent test.
RBC Red blood cell; red blood count.
RE Reticuloendothelial.
RES Reticuloendothelial system.
RF Rheumatic fever; rheumatoid factor.
RIA Radioimmunoassay.
RIF Receptor-inducing factor.
RIST Radioimmunosorbent test.
Ro See SS-A.
RPR Rapid plasma reagin.
RSV Respiratory syncytial virus.

S S value or sedimentation coefficient.
SAA Serum amyloid A.
SAC Staphylococcal protein A of Cowan I strain.
SBE Subacute bacterial endocarditis.
SC Secretory component.
SCID Severe combined immunodeficiency disease.
SCL-1 Antinuclear antibody found in scleroderma.
SD Serologically defined.
SFA Suppressive factor of allergy.
SIDS Sudden infant death syndrome.
sIgA Secretory IgA.
SIRS Soluble immune response suppressor.
SK-SD, SKSD Streptokinase-streptodornase.
SLE Systemic lupus erythematosus.
SMA Smooth muscle antibody.
SMAF Specific macrophage arming factor.
SNagg Serum normal agglutinator.
SpA Staphylococcal protein A.
SRBC Sheep red blood cells.
SRF Skin-reactive factor.
SRS-A Slow-reacting substance of anaphylaxis.
SS Systemic sclerosis.
SS-A Sjögren's syndrome antibody to RNA.
SS-B Sjögren's syndrome antibody to RNA.
SSPE Subacute sclerosing panencephalitis.
STS Serologic test for syphilis.

TA Transplantation antigens.
Tac T cell activation receptor.
TAF T cell-activating factor.
TATA Tumor-associated transplantation antigen.
TBM Tubular basement membrane.
Tc Cytotoxic T cells.
TCGF T cell growth factor (see IL-2).
TD Thymus-dependent.
Td Combined tetanus and diphtheria toxoid (adult type).
TdT Terminal deoxynucleotidyl transferase.
TEBG Testosterone-estrogen binding globulin.
TF Transfer factor.
Th Helper T cells.
Thy Thymus-derived.
TI Thymus-independent.
TL Thymic lymphocyte (antigen) on prothymocytes.
TLI Total lymphoid irradiation.
TMP Thymocyte mitogenic protein.
TNP Trinitrophenyl.
Tp Precursor T cells.
TPI *Treponema pallidum* immobilization.
TRA Thyrotropin receptor antibody.
Ts Suppressor T cells.

TSA Tumor-specific antigen.
TSab Thyroid-stimulating antibody.
TSH Thyroid-stimulating hormone.
TSI Thyroid-stimulating immunoglobulin.
TSTA Tumor-specific transplantation antigens.
TU Tuberculin units.
TX Thromboxane.

VCA Viral capsid antigen (of EBV).
VDRL Venereal Disease Research Laboratory.
VEA Virus envelope antigen.
V_H Variable domain of heavy chain.
VIG Vaccinia immune globulin
V_L Variable domain of light chain.
VLDL Very low density lipoproteins.

VSG Variable surface glycoprotein (of trypanosomes).
VZIG Varicella-zoster immune globulin.

WBC White blood cell; white blood count.

Z-DNA Methylated DNA coiled into a left-handed helix.
ZIG Zoster immune globulin.

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