Paparella: Volume I: Basic Sciences and Related Principles

Section 8: General Medical Principles

Chapter 36: Immunobiology

Norman T. Berlinger

The notion has been proffered that probably every major advance in the development of the knowledge of immunity has resulted from the presentation of a crucial experiment of nature to a uniquely prepared and receptive mind. Edward Jenner was impressed by the fact that a person who had contracted cowpox, a relatively harmless disease, did not develop smallpox. His disciplined powers of observation combined with his efforts to interpret this phenomenon experimentally led to the development of active immunization. In May, 1796, Jenner found a dairymaid, Sarah Nelmes, who had developed cowpox. On May 14, he inoculated a young boy, James Phipps, with material from the cowpox lesion on Sarah Nelmes' finger. James Phipps developed a low-grade fever and became mildly ill. Jenner inoculated the boy on July 1 with material from a smallpox lesions, but James Phipps did not develop the dreaded disease that was a leading cause of death at that time. Protection against smallpox had been conferred.

Louis Pasteur was a skillful experimenter endowed with a remarkable gift of observation. His observation that cholera organisms that had lost much of their virulence owing to suboptimal culture conditions were still capable of conferring immunity directly fostered the idea of attenuated vaccines. He thus succeeded in vaccinating sheep against anthrax and fowl against chicken cholera. His most spectacular effort was the obtaining of a weakened form of the rabies virus from the brain tissues of infected animals. On July 6, 1885, using such material, he saved the life of a young boy, Joseph Meister, who had been bitten by a rabid dog. The romance of immunobiology has so continued during the past 175 years - marked by von Pirquet's analysis that serum sickness was a form of immunologic injury, by Küstner's demonstration of the passive transfer of reaginic allergy, and by Fagraeus' demonstration that plasma cells synthesized antibodies in vitro.

The Cells of Immunity

In this vein, the interpretation of natural and laboratory experiments has yielded the knowledge that the functions of specific immunity are dually based. In studying the function of the bursa of Fabricius in the chicken, its role in humoral immunity was not fully appreciated until a fortunate laboratory accident occurred. Animals bursectomized immediately after hatching appeared incapable of manufacturing antibody. Subsequent experiments with rabbits and mice that had undergone neonatal thymectomy also revealed a state of impaired immunity, but the impairment was different from that observed in animals that had been bursectomized.

Analysis of the immunodeficiency diseases of children provided a striking parallel to these contrived laboratory experiments. Children with X-linked agammaglobulinemia are highly susceptible to infections caused by encapsulated pyogenic organisms, whereas they seem to resist fungal infections and certain viral infections quite well. Conversely, children

with the congenital third and fourth pharyngeal pouch syndrome (DiGeorge's syndrome) lack a thymus but often seem to have the capacity of normal immunoglobulin production. Analyses of these and other immunodeficiency diseases have resulted in the formulation of a distinct division of labor among the lymphocytes of the immune system into two main classes, B-cells and T-cells (Table 1).

Table 1. Functions of Lymphocytes

B-cells
- Defense against high-grade encapsulated pyogenic organisms and certain viruses by the elaboration of antibodies
- Formation of immunologic memory for anamnestic antibody response to above microbes

T-cells
- Defense against facultative intracellular bacteria, fungi, and certain viruses
- Formation of immunologic memory against above microbes
- Rejection of allografts
- Inhibition or destruction of tumor cells
- Helper cells for B-cell responses
- Suppressor cells for B-cell responses
- Generation of lymphokines.

B-Cells

It is generally accepted that a pluripotent hematopoietic stem cell exists during embryonic life that can undergo differentiative influences into erythroid, myeloid, or lymphoid cells. These stem cells are first apparent in the yolk sac and then migrate to other areas of hematopoiesis, ie, liver, spleen, and bone marrow, the latter being the major reservoir of stem cells during postembryonic life.

In the chicken, it is clear that the bursa of Fabricius, a hindgut lymphoepithelial organ, provides the necessary microenvironment for the differentiation into B-cells of stem cells that migrate there. Stem cells enter the bursa after about 2 weeks of embryonation and seem to undergo differentiation within 24 hours. Cells capable of synthesizing immunoglobulin-M (IgM) are found first, and, just prior to the time of hatching, cells appear that are capable of synthesizing IgG. Observations from animal experiments have led to a postulated scheme of events in the development of B-cells producing the various classes of immunoglobulins. It is probable that after a clone of cells that can produce IgM of a certain specificity has undergone a number of generations, some progeny undergo a gene expression switch to the production of IgG of the same specificity. It has been further postulated that B-cells capable of producing IgA are similarly derived from cells previously committed to IgG synthesis. In such a manner, a clone of B-cells can be generated that produce antibody of a single specificity, but among this clone are cells capable of producing this specific antibody as IgM, IgG, or IgA. Thus, when a subsequent encounter with antigen for this specificity occurs, the full defensive expression of the various classes of immunoglobulins can result.

The B-cells replicating in the bursa do so at an extremely high rate, perhaps in this way resulting in a swift generation of many clones of cells capable of producing antibodies

of many specificities. These cells populate the peripheral lymphoid tissue before the chick hatches. In humans, the identity of the bursa-equivalent remains enigmatic. However, the gut-associated lymphoid tissue, including Peyer's patches and the appendix, has been suggested as the possible equivalent, as has bone marrow. Once differentiated in the bursa-equivalent, B-cells take up residence as a rather sessile population of cells. They localize themselves in the outer cortex and medullary cords of lymph nodes; in the spleen, tonsils, and gut-associated lymphoid tissue; and in the bone marrow. Some B-cells circulate freely in the bloodstream and constitute about 5 to 25 per cent of the total circulating lymphocyte population.

B-cells and T-cells are indistinguishable under the light microscope, and other morphologic characteristics, such as the presence of surface microvilli detected by scanning electron microscopy, have not proved to be reliable markers for the identification of B-cells. Since B-cells bear immunoglobulin molecules on their surface membrane, they can easily be detected by staining techniques involving fluorochrome-tagged antisera to immunoglobulins. B-cells also have receptors for the crystallizable fragment (Fc) of IgG, for the third component of complement (C3), and for mouse erythrocytes. Techniques employing these reagents can readily identify these cells.

The primary function of the B-cell is to synthesize and secrete immunoglobulins once it has matured into a plasma cell. Each B-cell probably produces only one class or subclass of immunoglobulin, and there is no morphologic distinction among the B-cells capable of producing the various classes of immunoglobulins. The number of B-cells that can produce each class of immunoglobulin appears proportional to the percentage of each class of immunoglobulin within the body. Thus, the eventual role of the B-cell is to synthesize and secrete antibodies in response to infections with high-grade encapsulated pyogenic organisms such as *Streptococcus* or *Haemophilus influenzae.* It also seems important in the defense against certain viruses such as poliovirus or the virus of infectious hepatitis.

The exact mechanism for the induction of antibody formation is unknown, but when an antigen enters the body, it most likely interacts with a homologous antibody on the surface of a B-cell. A necessary prior step may be the phagocytosis and processing of the antigen by a cell of the monocyte-macrophage series and the subsequent presentation of the antigen, perhaps in a more immunogenic form, to the B-cell. The B-cells that have the homologous antibody on their surface, especially of the IgM class, are then driven to a terminal stage of differentiation to become antibody-secreting plasma cells. This terminal step takes place in the spleen and in the medullary cords and outer cortex of lymph nodes. The B-cell undergoes blast transformation and cell division to increase the numbers of final antibody-secreting plasma cells, which can be identified by the pyroninophilic cytoplasm and cartwheel chromatin pattern and the presence of intracellular antibody. These plasma cells live approximately 2 to 3 days and secrete antibody of a specificity corresponding to the antigen. Concurrent with the generation of plasma cells is the generation of memory cells. These cells can live from 3 to 20 years and appear to provide the necessary link between the initial encounter with the antigen and subsequent encounters.

The pattern of antibody production during the first contact with antigen (primary immune response) is different from a subsequent contact with the same antigen (secondary or anamnestic response). The primary immune response is sluggish, perhaps reflecting the necessary clonal expansion of B-cells homologous for this antigen, and primarily yields

antibody of the IgM class. The secondary immune response is rapid, perhaps reflecting the influence of memory cells, and primarily yields IgG. Whatever the type of response, the heavy and light chains of the antibody molecule are synthesized in separate polyribosomes of the plasma cell and are assembled in the membrane-bound endoplasmic reticulum. The entire process from synthesis to secretion requires only about 30 minutes.

In addition to their role in defense against foreign invaders, antibody molecules seem to exert certain regulatory influences. Once formed, antibody appears to operate in a feedback mechanism, suppressing the further formation of antibody and thus probably holding the immune response in check. Antibody may also act in positive fashions. Preformed antibody of a certain specificity may compete with B-cells of the same specificity for an invading antigen and thus force the formation of antibody of even higher affinity. Also, antibody of a certain specificity could combine with the antigen and force the formation of new antibody to a different specificity on the same antigen, thus resulting in an eventual immune response capable of addressing numerous specificities on the same antigen.

Disorders of antibody production indicate the various stages in B-cell development at which errors can occur. Patients with X-linked agammaglobulinemia may totally lack B-cells, indicating a failure of B-cell development. Other individuals with the same disease may possess B-cells but are still agammaglobulinemic, denoting an inability of the B-cell to differentiate into a mature immunoglobulin-secreting plasma cell. Individuals may demonstrate a selective deficiency of IgA, pointing out a defect in the ability of only those B-cells committed to synthesizing IgA to differentiate into plasma cells. Malignancies of the lymphoid apparatus likewise demonstrate that malignant adaptation may occur at various stages in B-cell development. Chronic lymphocytic leukemia appears to be a malignant expansion of the B-cell compartment, usually of those cells committed to synthesizing IgM; yet still up to 50 per cent of patients with this condition develop extreme hypogammaglobulinemia. Multiple myeloma is a dyscrasia of the end-stage of differentiation of the B-cell, namely, the plasma cell. Patients with this disorder demonstrate an excess of both an immunoglobulin in their serum and Bence Jones protein (the light chain of the immunoglobulin molecule) in their urine. The excess serum immunoglobulin may belong to any one of the five major heavy chain classes, IgG, IgA, IgM, IgE, or IgD. Waldenström's macroglobulinemia is a plasma cell dyscrasia involving only those plasmacytes capable of synthesizing IgM. Heavy chain disease is a rare form of plasma cell dyscrasia involving cells producing IgG heavy chain or IgA heavy chain and a concomitant inability to assemble cells with light chains to form complete immunoglobulin molecules.

T-Cells

Fetal stem cells may also migrate to the thymus. This organ develops from the ectodermal-endodermal junctions of the third and fourth pharyngeal pouches and then descends to lie in the anterior mediastinum over the roots of the great vessels. Late in the first trimester of gestation, fetal hematopoietic stem cells originating from the yolk sac or liver enter the thymus and undergo a rapidly marked proliferation. The proliferating cells migrate from the cortex of the lobule inward to the medulla, from which they are ultimately discharged to the peripheral lymphoid tissue.

During the phase of proliferation, the cells are differentiated into immunocompetent

lymphocytes. This differentiation occurs within the microenvironment provided by the epithelial component of the thymus. There is growing evidence that the differentiative influence is a humoral factor derived from the thymic epithelium. During differentiation in the mouse, these lymphocytes acquire a surface marker known as the teta-isoantigen; the human equivalent remains unknown. In addition to the direct influence exerted within this gland, the thymus also has a clearly separable function of maintaining and expanding the peripheral population of post-thymus cells. If experimental neonatal animals are thymectomized, the already peripheralized T-cells are not maintained, and the functions of T-cell-dependent immunity are gradually lost. This situation can be circumvented by implanting a thymus situated in a cell-impermeable chamber into a thymectomized animal.

The post-thymic T-cells take up residence in the white pulp of the spleen, the deep cortical regions of lymph nodes, Peyer's patches, and the bone marrow. Unlike B-cells, T-cells are a rapidly recirculating population of lymphocytes, likened to a flying squadron. About 70 to 90 per cent of lymphocytes circulating within the intravascular compartment are T-cells. The control of lymphocyte flow after leaving the thymus probably depends on surface membrane molecular characteristics because treatment of these cells with certain enzymes causes the cells to wander aimlessly and lose their homing instincts for peripheral lymphoid tissue.

Morphologic identification of T-cells is not possible. Rather, they possess certain characteristic surface molecules and receptors. In the mouse, these cells possess the TL marker while in the thymus. In vitro, sheep red blood cells can bind to human T-cells, giving rise to the so-called T-rosette. Numerous investigations have led to the conclusion that human lymphocytes that form such rosettes are thymus-derived cells. Such cells cannot be stained with antisera to heavy chains of immunoglobulins, and they lack receptors for C3. Furthermore, rosetting cells and cells that bear B-cell markers compose approximately 100 per cent of the lymphocytes circulating in the blood.

Experiments involving the neonatal thymectomy of animals have elucidated the major functions of T-cells. In short, T-cells are responsible for delayed hypersensitivity, a term that is now often equated with the term cellular immunity or cell-mediated immunity. T-cells provide the defense against many viruses, fungi, and facultative intracellular bacterial pathogens such as tubercle or lepra bacilli. These functions are reminiscent of the classic demonstration in 1942 by Landsteiner and Chase that tuberculin reactivity could be transferred to normal individuals by using peritoneal cells from sensitized donors but not by using antibody-containing serum. Likewise, mousepox infections can be controlled by immune spleen cells that are passively transferred to an infected host but not by hyperimmune serum. Treatment of the spleen cells with an antiserum directed against T-cells abrogates the protective effect. Patients with deficient humoral immunity, eg, hypogammaglobulinemia, can develop measles and recover. However, patients with deficient cell-mediated immunity (CMI) will acquire a giant cell pneumonia and die. Children with deficient CMI who are inadvertently inoculated with smallpox vaccine will develop a progressive vaccinia that cannot be controlled by the administration of hyperimmune vaccinia immune globulin.

T-cells also play a major role in the rejection of organ transplants (allografts). This concept was originally based on the observation that the transfer of sensitized cells from an immune animal results in the elimination of transplanted tissue, whereas immune serum does

not show this capacity. This formulation is not absolute, however, because patients with deficiencies of humoral immunity can show less vigorous rejection of transplanted tissue, suggesting some sort of role for antibodies.

Since malignant tissue acquires new cell surface antigens, it is probable that the function subserved by T-cells constitute an important mechanism for the control of tumor growth. Neonatal thymectomy of mice renders them more susceptible to malignant conversion by polyoma virus. CMI wanes with advancing age, as measured by reactivity to purified protein derivative (PPD) or by the ability to become sensitized to dinitrochlorobenzene. It may be more than coincidental that the incidence of malignancy in the general population increases dramatically with advancing age. The immunosuppressive measure employed to ensure successful renal transplantation have resulted in a risk of malignancy in these patients approximately 80 times that found in the general population. In addition, certain immunodeficiency diseases, such as the Wiskott-Aldrich syndrome, severe combined immunodeficiency, and ataxia-telangiectasia, have an associated incidence of malignancy of 6 to 10 per cent.

T-cells also serve important roles in interacting with B-cells to allow these latter cells the full expression of their function in body economy. The importance of the cooperative interactions between these two cell types was illustrated by hapten-carrier studies. When T-cells recognized a carrier moiety, the result was production of antibody by B-cells to the hapten linked to the carrier. Thus, some T-cells that serve such a function are known as helper cells, because their participation is necessary to yield an effector B-population. Sheep red blood cells constitute another antigen used experimentally to demonstrate the requirement, in some instances, for T-cell helper activity in antibody responses. Lethally irradiated mice will produce antibody to sheep red blood cells only when their T- and B-cell populations are both restored but not when one or the other population is restored. The situation is more difficult to assess in humans, but some cases of isolated thymic deficiency do not show adequate antibody formation. Antigens have been artificially classified as thymus dependent or thymus independent, according to whether or not the participation of T-cell helpers is necessary for an antibody response.

T-cells may also control antibody production in a negative fashion, and such T-cells are known as suppressor cells. These cells can exert their effects in a specific manner. For example, thymocytes from rats inoculated with bovine gamma globulin (BGG) can, on adoptive transfer to normal rats, specially suppress the antibody response to BGG. Also, the adoptive transfer of immune cells from mice tolerant to a particular antigen can confer specific nonreactivity on mice. There also is evidence to suggest that some of the effects of suppressor T-cells are exerted in a nonspecific manner. Desensitization of guinea pigs to an antigen can result in anergy to other antigens to which the animals had been immunized but not desensitized. This effect could not be explained by simple deletion of the lymphocytes capable of responding to the antigens, they could respond to the antigens in vitro. At present, the models of T-cell suppression of antibody production must be tested in multiple situations before their validity to immunologic homeostasis can be assessed. However, suppressor T-cell function has now been implicated in the genesis or maintenance of some forms of human hypogammaglobulinemia.

The specificity characteristic of the activation of T-cells appears to be a direct function

of their cell surface antigen-binding receptors, just as is the case with B-cells. There is much dispute as to whether the antigen-specific receptor on the surface of T-cells is an immunoglobulin molecule of some kind, but the available evidence does not favor this view. The nature of the antigen receptor remains completely obscure, but it appears that the T-cell receptor does not have as fine a discriminatory capacity as does the B-cell receptor.

Neutrophils

In primitive forms of life, phagocytic cells (Table 2) serve the function of engulfing and processing food. In more advanced forms of life, Metchnikoff has shown that phagocytes engulf foreign microbial invaders. These phagocytic cells can be divided into a circulating group, ie, neutrophils, eosinophils, and monocytes, and into a fixed group of cells, which includes the tissue histiocytes of the spleen, liver (Kupffer's cells), lungs, lymph nodes, and brain (microglia).

Table 2. Functions of Phagocytes

Neutrophils
- Phagocytosis of microbes, particularly bacteria and fungi
- Intracellular killing of microbes

Macrophages (monocytes)
- Phagocytosis of microbes, particularly bacteria and fungi
- Intracellular killing of microbes
- Defense against extracellular parasites (?)
- Inhibition and destruction of tumor cells
- Interferon production
- Antigen processing and antigen presentation
- Induction of T-cell proliferation
- Suppression of T-cell proliferation
- Induction of some B-cell antibody responses.

During embryonic life, granulocytopoiesis begins in the liver, but the main focus of activity shifts to the bone marrow late in the second trimester. Myeloblasts are the first recognizable granulocyte precursors and differentiate into promyelocytes and myelocytes, these last two form having recognizable granules. Segmentation of the nucleus occurs as the cell undergoes differentiation from the metamyelocyte to the mature form. About 10 days are required for all the differentiative steps from stem cell to granulocyte to take place so that the mature granulocyte can be released into the circulation. Of the granulocytes in the intravascular compartment, half circulate freely and the remainder are marginated along the walls of small blood vessels. The half-life of an intravascular neutrophil is estimated to be 6 hours. The turnover of neutrophils is so rapid that the daily degradation is estimated to be between 50 and 100 mL of packed neutrophils.

Patients born with congenital neutropenia usually show an inability to raise their circulating neutrophil numbers by mobilizing marginated cells after epinephrine stimulation or by mobilizing bone marrow neutrophils after infusion of hydrocortisone. Most frequently, these patients demonstrate a maturation arrest in the bone marrow at the promyelocyte stage. However, the pathogenesis of congenital neutropenia is not the same in all cases, since a child

with this disease has been reported who can form mature neutrophils in soft agar culture in vitro. A defect in the microchemical environment available to stem cells committed to granulocyte differentiation must be postulated, as must also an inhibitor of granulocyte precursor maturation that is operative in vivo but not in vitro.

One of the most striking properties of neutrophils is their ability to move themselves on the walls of blood vessels, in tissues, or on foreign laboratory surfaces. During locomotion, neutrophils send out pseudopods, but there is no cytoplasmic streaming, and the granules maintain their position unchanged. Random motility has been described for neutrophiles, in that they tend to change direction approximately every 20 to 40 microns. However, directed locomotion, or chemotaxis, has also been described when the random movement abruptly changes to straight line movement toward the object of phagocytosis. The chemotactic stimuli seem to be components of the complement system, especially the low molecular weight fragments of the third and fifth components, C3a and C5a, which are made available when antibody combines with antigen and subsequently fixes complement.

Clinical abnormalities of neutrophil chemotaxis have now been described. Abnormalities involving defects intrinsic to the neutrophil are seen in the Chédiak-Higashi syndrome, the "lazy leukocyte" syndrome, diabetes mellitus, rheumatoid arthritis, and chronic mucocutaneous candidiasis. Abnormalities involving the defective or deficient generation of chemotactic stimuli from serum constituents are seen in patients with C5 dysfunction and C3 hypercatabolism. Finally, children with recurrent infections can have chemotactic defects associated with neither of the aforementioned problems but seem instead to possess circulating inhibitors of the chemotactic response. In all three defects, the precise contribution of impaired chemotaxis to the greatly increased susceptibility to infection is unknown.

Approximately 30 x 10^9 neutrophils, ready to phagocytose foreign bacteria, are in the circulation of a healthy adult. At an area of inflammation or injury, the neutrophils first adhere to the capillary wall and then migrate into the tissues. When they encounter the foreign material, they send out thin filamentous processes around it that eventually join, thus isolating the foreign material in an intracellular phagocytic vacuole. The granules of the neutrophil move toward the phagocytic vacuole, and their contents are discharged into it. The entire process of phagocytosis can be completed within 2 minutes. Bacteria can be cleared whether or not a pre-existing state of immunity to the particular organism is extent, and, as such, the phagocytic function of neutrophils can be deemed nonspecific in the true immunologic sense. However, the bacteria must generally be modified by serum components before phagocytosis ensues. These serum components that prepare bacteria for phagocytosis are known as opsonins. One of the most effective opsonins is specific antibody to the bacterium, and evidence has been presented that the neutrophil possesses a receptor for the Fc portion of opsonic IgG, although the trigger mechanism for phagocytosis once the Fc portion has joined with the receptor is unknown. Other serum factors without the specificity of antibody can function as opsonins. The complement system plays a significant role in the opsonization of bacteria, but other heat-stable and heat-labile serum opsonins exist whose chemistry is unknown. The Chédiak-Higashi syndrome is interesting in the context of phagocytosis in that in this disorder the neutrophils are engaged in a significant degree of autophagic activity, resulting in many cytoplasmic inclusions and an accelerated rate of neutrophil turnover.

Once the granules have spilled their contents into the phagocytic vacuole, the process

of intracellular bacterial killing may begin. Chemically, this is a complex process that has been only partially elucidated. Some early attention was focused on granular cationic proteins called leukin and phagocytin, which appeared capable of destroying many gram-positive and certain gram-negative species. More recent attention has focused on two enzymes, muramidase and myeloperoxidase. Muramidase hydrolyzes cell wall constituents of some gram-positive bacteria, but its narrow spectrum suggests that other factors participate. Klebanoff developed a substantial body of evidence indicating that iodine or other halogens in the presence of hydrogen peroxide and myeloperoxidase activity lead to the halogenation of bacteria, the direct cause of their destruction. Others have proposed that the myeloperoxidase-hydrogen peroxide-halide system leads to the production of bactericidal aldehydes.

Chronic granulomatous disease (CGD) is an inherited neutrophil defect associated with markedly deficient intracellular bacterial and fungal killing. As glucose is utilized by neutrophils during intracellular killing, hydrogen peroxide normally accumulates. Patients with CGD effectively handle organisms that produce hydrogen peroxide but handle poorly those catalase-positive organisms that can break down hydrogen peroxide. This combination of facts has led to the postulation that a central defect in patients with CGD is that their neutrophils do not produce hydrogen peroxide efficiently or at all. Therefore, myeloperoxidase cannot function appropriately for the generation of final microbicidal products or events.

Monocytes and Macrophages

Cells of this series arise from stem cells in the bone marrow and then circulate in the blood as monocytes. Their life span in the circulation appears to be from 3 to 6 days. Unlike neutrophils, these circulating cells are not end-stage cells. They migrate out of the intravascular compartment into tissues at the site of antigenic challenge, where they undergo further differentiation into macrophages. These are large cells with an eccentrically placed reniform nucleus and abundant cytoplasm containing lysosomes, vesicles, and a cytoplasmic machinery capable of secreting enzymes with a wide range of activities.

Until recently the role of macrophages in efferent immunity has been emphasized. These cells possess the capacities of micropinocytosis, macropinocytosis, and phagocytosis, but they are less efficient killers of bacteria than are neutrophils. Nevertheless, they still constitute an important line of defense against viral and facultative intracellular bacterial infections. Macrophages can bind antigen to their surfaces either directly or indirectly if the antigen is coated with antibody or with antibody and C3, since these cells possess Fc and C3 receptors. After binding, phagocytosis follows and is capable of being carried out in a segmental fashion. Phagocytosis at one site does not initiate phagocytosis at other sites on the macrophage membrane, thus allowing other cooperating immunocompetent cells to remain in intimate contact with the macrophage. The phagocytic vacuoles then fuse with lysosomes, and destruction of the ingested material begins. There is a certain lack of specificity in this entire process in that macrophages activated in the course of an immune response to bacillus Calmette-Guérin (BCG) infection will destroy not only BCG but also unrelated microorganisms such as *Listeria monocytogenes.* Whether or not macrophages significantly take part in the defense against extracellular metazoan parasites is still unclear.

Macrophages appear to play an important efferent role in the inhibition and destruction

of tumor cells. In experimentally induced animal tumors, it has been shown that the macrophage content of these tumors varies considerably and is inversely related to their capacity to metastasize. A number of reports indicate that this may also be true for human tumors. The mechanism underlying this phenomenon is unknown. In vitro studies have disclosed that macrophages can exert profound effects upon tumor cells, and recent evidence suggests this may occur because of the elaboration of a cytostatic substance by the macrophage. On contact with the tumor cell, translational movements of activated macrophages are increased considerably, and macrophages appear to make repeated contacts, lasting several hours, with the tumor cell. Macrophages may also kill tumor cells by nonphagocytic mechanisms. The nature of this process is unclear, but some evidence exists suggesting that during the periods of macrophage-tumor cell contact, macrophage lysosomal constituents are directly injected into the tumor cell. This capacity to kill tumor cells is in direct contradistinction to the effects of macrophages on normal non-neoplastic cells. When normal cells are proliferating rapidly, macrophages can inhibit the growth of these cells, but no cellular destruction is observed. Thus, macrophages can affect the growth of normal cells, but they can affect the viability of tumor cells, and this may be a function of the altered surface constituents of the tumor cells. In addition to their lytic capacity, macrophages may also kill tumor cells by phagocytosis, some reports suggesting that the macrophage does so in piecemeal fashion.

The effector role of macrophages in the destruction of viruses is enigmatic; for some phagocytosed viruses are destroyed or their replication inhibited, whereas other viruses persist alive within the macrophage and can continue to spread in the host. Interestingly, macrophages have been shown to have the capacity for production of interferon, a substance with potent antiviral properties, and these cells could represent the major source of interferon appearing in the serum.

Past emphasis has relegated cells of the monocyte-macrophage series to a status of mere scavenger in effector immunity. This position is no longer tenable, for recent investigations have placed the macrophage in a central and crucial role in the induction processes of afferent immunity. Macrophages are required in vitro for the activation of T-cell proliferation and mediator production induced by soluble protein antigens, cell-associated antigens, and chemically modified cell surface glycoproteins. In the mouse, macrophages also appear necessary for the induction of primary B-cell antibody responses and for the generation of T-helper cells.

This functional demonstration of a macrophage requirement has not yet advanced to the stage at which mechanisms have been defined. However, it had been observed earlier that antigens could persist in macrophages for long periods of time, in contradistinction to neutrophils. It seems possible that this extended intracellular residence reflects macrophage processing of antigen so that it may subsequently be presented to lymphocytes. In this regard it is interesting that mice that are poor responders to certain antigens show evidence of a more complete intracellular macrophage degradation of the antigen, although there are notable exceptions. Such a process suggests that decreased efficiency of macrophage presentation of antigen to lymphocytes may be a logical explanation. It appears that macrophages can present antigen on their surfaces in two ways. One is antigen that has not been phagocytosed but is only bound to the macrophage surface. The other involves phagocytosis; intracellular processing, possibly with coupling of the antigen to macrophage ribonucleic acid (RNA); and

exteriorization of the RNA-antigen complex back onto the surface of the macrophage as a super-antigen. Binding of lymphocytes to macrophages bearing such antigenic signals has been observed in vivo, although the significance of such binding to macrophage-lymphocyte interactions is unclear. However, in vitro evidence suggests that such physical interaction is a necessary antecedent to the antigen-initiated proliferation of lymphocytes. Thus, macrophages could be responsible for initiating many primary and secondary immune responses, for enhancing the immunogenicity of some antigens, and even for regulating lymphocyte responses to certain antigens. This most exciting latter possibility stems from observations that for macrophages and T-lymphocytes to interact appropriately, they must share some common cell surface determinant specified by the major histocompatibility complex within which lie immune response (Ir) genes capable of controlling immune responses.

Clinical disorders of macrophage function are just being defined. In murine systems, the undue susceptibility of newborns to infections with herpes simplex viruses and cytomegalovirus can be obviated with administration of adult macrophages, suggesting an immaturity of macrophage function in very young animals. In humans, the striking clinical similarity of the virulence of these organisms at younger ages is an intriguing parallel, but immature macrophage function in the human neonate awaits description. Macrophages from patients with the Wiskott-Aldrich syndrome, chronic mucocutaneous candidiasis, or Chédiak-Higashi syndrome can show deficient chemotactic responses. Macrophages from patients with chronic granulomatous disease are similar to their neutrophils in that intracellular killing is defective but phagocytosis is normal.

Clinically, the macrophage appears to be intimately involved in the immune response to malignancy. Patients with a variety of cancers can show deficient chemotactic responses to the complement component C5a or a lymphocyte-derived chemotactic factor (LDCF). Interestingly, deficient chemotactic responses correlate with early recurrences of melanoma. A more central role for the macrophage in malignancy derives from studies of macrophage modulation of lymphocyte responses. Mice bearing spontaneous mammary adenocarcinomas, methylcholanthrene-induced rhabdomyosarcomas, or tumors induced by the Moloney sarcoma virus possess a population of cells belonging to the monocyte-macrophage series that are capable of suppressing the responses of lymphocytes to nonspecific mitogens or the allogeneic cells in mixed leukocyte culture (MLC). These observations have been extended to human cancers, particularly epidermoid cancer of the head and neck region. Such a finding may explain the decreased cellular immunocompetence so often seen concomitant with malignancy. This finding may also be useful clinically in prognosis and in individualizing therapy, since it is known that decreased cellular immunity in patients with these cancers correlates with prognosis and the response to conventional modes of therapy.

The Molecules of Immunity

Immunoglobulins

Immunoglobulins are proteins that are produced predominantly by plasma cells and by some lymphocytes and are present in the serum, tissues, and exocrine secretions. They are primarily gamma globulins but also include related proteins such as myeloma proteins, Bence jones proteins, and naturally occurring subunits of immunoglobulins. Immunoglobulins that

possess demonstrable antigen-combining activity are called antibodies, but no antibody activity has been described for the related groups of proteins just mentioned. Immunoglobulins are the mediators of humoral immunity (Table 3).

Table 3. Functions of Immunoglobulins

Mediators of humoral immunity
Feedback inhibition of antibody formation
Probable receptor for antigen on B-cell surfaces (IgD?)
Fixation of complement (IgM and IgG)
Passive immunity for the fetus by placental transport (IgG)
Opsonization (IgM and IgG)
Virus neutralization (IgM, IgG, and secretory IgA)
Blocking antibody in allergic desensitization (IgG)
Natural isohemagglutinins (IgM and IgG)
Protection of mucosal surfaces (secretory IgA)
Mediator of immediate hypersensitivity (IgE)
Prevention of autoantibody formation (IgA?)
Defense against parasite infestations (?).

The gamma globulins are a heterogeneous group of proteins divided into major classes depending upon the nature of the chains composing the molecule. The basic structure is that of two identical heavy chains and two identical light chains linked together by disulfide bridges and weak noncovalent bonds. There are two antibody combining sites on this basic structure, each formed by a portion of the heavy chain and light chain at the end of the molecule. Heavy chains are of five types, gamma, alpha, mu, delta, and epsilon, and in part determine the class to which the immunoglobulin belongs, IgG, IgA, IgM, IgD, or IgE, respectively. Light chains are of two types, kappa and lambda. For example, an IgG molecule possessing two gamma heavy chains may possess either two kappa light chains or two lambda light chains. Thus, there are 10 immunoglobulin classes, depending on the nature of the light chains linked to the heavy chains. Subclasses have been identified in normal serum, each having unique biologic and metabolic properties. IgG, IgD, IgE are monomers of the basic structural unit of two heavy chains and two light chains. IgM is a pentamer, whereas IgA exists as a monomer in the serum and as dimers and trimers in the secretory form.

The plasma cell, the end-stage differentiated form of the B-cell, synthesizes the majority of immunoglobulin on the ribosomes of the endoplasmic reticulum. Since the plasma cell is relatively short lived, immunoglobulin is not stored but can temporarily accumulate in inclusions called Russell's bodies. Heavy chains and light chains are synthesized separately and are combined prior to release into the serum, although the stimulus for immunoglobulin release is unknown. Immunoglobulin is broken down in the intestinal lumen and in the reticuloendothelial system, especially that of the liver. Immunoglobulin destruction can, of course, occur within phagocytic cells that have engulfed immunoglobulin-coated particles.

IgG

This immunoglobulin makes up about 80 per cent of serum immunoglobulins. IgG antibodies are of high affinity and fix complement well. They make up the majority of the

body defenses against bacteria, viruses, and toxins. IgG is the only class of antibody to cross the placenta to the fetus and provides the neonate with some passive immune function for as long as 6 months. If an initial encounter with an antigen results in an IgG response, immunologic memory is established, and a subsequent response to that same antigen (anamnestic response) takes place primarily with IgG antibodies. Allergic desensitization evokes the production of blocking antibodies of the IgG class, which can combine with the allergen before it combines with IgE.

IgM

Antibodies of this class are also unusually avid and fix complement well. They can act as efficient opsonins and can also agglutinate antigenic particles, probably because of the 10 potential combining sites on the pentameric molecule. This class of antibody is the first formed after an encounter with antigen, and if only an IgM response occurs, no immunologic memory is established. IgM antibodies are the major defense against gram-negative bacteria and constitute most of the antibodies to A and B substances (isoagglutinins). IgM is the main immunoglobulin class that can be synthesized by the fetus, although usually little is synthesized in utero. However, intrauterine infection can provoke fetal synthesis of IgM, especially as a consequence of intrauterine infections such as rubella, syphilis, toxoplasmosis, and cytomegalic inclusion disease. Elevated levels of IgM in cord blood or neonatal serum suggest intrauterine infection. However, infections can occur in the absence of an antibody response, and, therefore, normal IgM levels do not preclude the possibility of intrauterine infection. Elevated cord levels of IgM may be false-positive results due to leakage of maternal blood to the fetus.

IgD

This class of immunoglobulin is present in serum in very small quantities. Controversy exists as to whether this class possesses antibody activity, and no definite role has yet been assigned to IgD. Since some B-cells that bear surface IgM have also been found to bear surface IgD, some wish to ascribe the role of antigen receptor to IgD. This remains to be established.

IgA

This class of immunoglobulins is the second most abundant in the body and possesses a wide range of antiviral and antibacterial activities; however, it provides no known unique defense mechanism. IgA does not fix complement, and individuals who have an isolated deficiency of serum IgA antibodies do not show an inordinate propensity to infections. One postulation is that IgA antibodies can combine with antigens from damaged tissues or organs and consequently obviate antibody responses to these antigens of the IgG or IgM classes, classes that could exert harmful autoimmune effects because of their high avidity and ability to fix complement. In this regard, it is interesting that individuals who lack IgA have a high incidence of autoantibodies. Selective IgA deficiency is probably the most common form of the immune deficiency state, its incidence being estimated at about 1 in 500 individuals. Patients with selective IgA deficiency have been found with the greatest frequency among individuals with autoimmune disorders, hypogammaglobulinemia, and allergies. This latter association is provocative in that the majority of blocking antibodies in external secretions of

allergic individuals are of the IgA class. It is possible that the deficiency of blocking IgA antibodies in these patients' secretions could result in an increased incidence of allergic symptoms. Patients with selective IgA deficiency and allergies have more gastrointestinal tract complaints and more frequent respiratory tract infections.

IgA also occurs in a secretory form in external secretions. Here, it is predominantly a dimeric form with the addition of a glycoprotein moiety of epithelial origin known as secretory piece or secretory component (SC). IgA-producing cells are located preferentially along glandular structures, and their presence there is not dependent on inflammation. This predominance of IgA-producing cells is not observed along mucosal surfaces having few glandular structures, such as the gingiva. The origin of these cells is not yet known, but their local proliferation is probably due to the antigenic stimulation provided by the microbial flora to which mucous membranes are constantly exposed. This mechanism seems clear for the respiratory and digestive tracts, but the mode of cellular stimulation is unclear for glandular structures such as the lacrimal or salivary glands, which are less intensely exposed to microbes.

Secretory IgA is synthesized locally in these immunocytes, but its exact mode of transmission through the glandular epithelium to the external environment is unknown. Intercellular and intracellular transport steps both seem operative. That SC is synthesized by cells other than the IgA-producing immunocytes, probably by glandular epithelial cells, is supported by the observation that SC is present in IgA-deficient secretions. The site of conjugation between SC and IgA dimers is unknown. Other classes of immunoglobulins find their way into the external secretions probably by passive diffusion of serum immunoglobulins through epithelium and by exudation potentiated by inflammation.

Secretory IgA is the first line of immune defense against the external environment. Its prominent local synthesis and marked stability to the adverse effects of many external body fluids qualify it well for this role. The protective effect of secretory IgA antibodies is clear only for superficially proliferating viruses. Thus, secretory IgA antibodies can neutralize rhinovirus, poliovirus, influenza virus, and parainfluenza virus. In fact, resistance to reinfection by these agents generally shows a better correlation with secretory antibody titer than with serum antibody titer. The role of secretory IgA antibodies in defending against bacterial microbes is less clear. It appears the secretory IgA can exert rather good opsonizing activity and agglutinating activity. Thus, its primary role against bacteria may be the simple combination with them, with subsequent trapping of these clumps in the continuously removed mucin coat of mucous membranes or with subsequent removal of phagocytic cells. Secretory IgA antibodies do not bind complement in the classic pathway and, therefore, cannot exert appreciable bacteriolytic activity. However, it has recently been suggested that complement can be activated by the alternate pathway (to be discussed).

IgE

IgE was practically coincidentally discovered by the Ishizakas in 1966 and by Johansson and Bennich in 1967. These two groups of investigators then showed that the immunoglobulins that each had discovered were in fact identical. These studies marked a turning point in the history of immediate hypersensitivity, for almost immediately the field of allergy, which had previously been regarded with some suspicion by immunologists, gained

its rightful aura of respectability. The reaginic, or IgE, antibodies were soon found to be unlike the other classes of antibodies in many ways. They were heat sensitive, they were nonprecipitable, they did not cross the placenta, they did not fix complement in the usual manner, and they were not detectable until recently with any conventional immunologic method.

IgE binds to circulating blood basophils and to tissue mast cells by its Fc portion (the crystallizable portion of the two heavy chains most distant from the light chains). Exposure of these sensitized cells to the particular allergen for which the IgE has specificity leads to release of mediators. E myeloma proteins (a homogeneous IgE produced by the malignant expansion of a clone of B-cells committed to IgE synthesis) cannot mediate immediate hypersensitivity because the particular allergen for which they have specificity is unknown. However, they are capable of blocking the Prausnitz-Küstner reaction, presumably by saturating the receptor sites on mast cells for native IgE.

The clinical manifestations of immediate hypersensitivity are caused by potent pharmacologic mediators released by the target, or sensitized, cells. Blood basophils and tissue mast cells contain most of the body's histamine. This substance dilates small blood vessels, increases capillary permeability, contracts bronchial and other smooth muscle, and stimulates mucous glands. It is the mediator of the triple response of Lewis and is responsible for many wheal and flare reactions. Thus, it plays an important role in allergic rhinitis and asthma. Serotonin is somewhat effective in producing bronchoconstriction, but its role in human allergic disorders is thought to be negligible at best. Anaphylatoxin is a serum-derived substance that produces physiologic alterations similar to the effects of histamine. It appears that two components of the complement system, C3 and C5, fulfill the general criteria of anaphylatoxin. Their effects may be exerted indirectly by their ability to promote histamine release. Anaphylatoxin may promote urticaria and serum sickness and may also be involved in atopic disorders. Kinins (especially in this context bradykinin and kalidin) are pharmacologically active polypeptides generated from serum components by a number of enzymes that can cause vasodilation, increased vascular permeability, contraction of nonvascular smooth muscle, and pain. They have been implicated in anaphylactic shock, asthma, and certain other allergic disorders. The slow-reacting substance of anaphylaxis (SRS-A) is one of a group of lipid-soluble substances that can cause contraction of bronchial smooth muscle. Its action is slower and more prolonged than that of histamine, hence the name. By a mechanism involving IgE, SRS-A can be released from human lung tissue or peripheral blood leukocytes, although its parental material or cellular source remains unknown.

The most important biologic property of IgE is its ability to sensitize homologous tissue. E myeloma protein injected into monkeys can be detected on mast cells in the skin, omentum, small intestine, and bronchi. A question arises concerning how many IgE molecules are present on the surface of target cells. In both normal and atopic individuals, basophils can bind 10.000 to 40.000 molecules of IgE. However, it is quite clear that the number of cell-bound IgE molecules has no correlation with serum IgE concentration. This is an important clinical point, for it demonstrates that elevated serum levels of IgE may not be a foolproof indicators of the presence or severity of allergic symptoms. Rather, the ability to sensitize basophils and mast cells with IgE could be more reliable. It appears that the greater the cell-bound IgE, the smaller the optimum stimulus of allergen necessary to trigger histamine

release from the sensitized cells. It should be noted, however, that the number of cell-bound IgE molecules bears no relationship to the amount of histamine released by the minimal optimum stimulus. This is determined by the enzyme systems in the cells that regulate biochemical processes for the release.

Genetic controls seem to operate to determine whether reaginic antibody will be produced in response to an antigen. In mice, the murine histocompatibility system (H-2 system) is inextricably linked to certain disease processes. Susceptibility to the Gross leukemia virus, to vaccinia virus infection, and to methylcholanthrene inducibility of tumors is directly related to the genotype of the mouse at or near the H-2 loci, although the role of the H-2 genes is not clear. Likewise, the capacity to produce reaginic antibody in the mouse is governed by two distinct genetic controls. One control appears to be an independently segregating autosomal locus not yet mapped. The other is a single autosomal locus closely linked to the H-2 system. Both are operative to define genetically whether or not a given mouse will mount a reaginic immune response to a given antigenic stimulus, in this case, benzylpenicilloyl bovine gamma globulin. Data from breeding experiments show this responder trait to be autosomally dominant. For humans, a number of investigators all showed independently that sensitivity to ragweed antigen E or to ragweed allergen RA-5 was associated with a certain histocompatibility phenotype, notably HLA-A7. Also proffered has been association between childhood asthma and both HLA-A1 and HLA-B8. Although rudimentary, the understanding of the genetic controls operative within the human major histocompatibility complex (MHC) may have direct impact in determining those individuals at risk for demonstrating allergic disease

The production of IgE is under a type of autogenous regulation similar to that for other classes of immunoglobulins. The production of IgE appears to be regulated by T-cells. Immunosuppressive measures such as thymectomy or the administration of sublethal x-radiation or certain immunosuppressive drugs result in enhancement and prolongation of IgE formation in animals. This suggests that T-cells keep IhE formation at "appropriate" levels by an active process of suppression. Interestingly, patients with Hodgkin's disease, which is believed to involve an abnormality of T-cells, often show an elevation of IgE.

The bulwark of the immunotherapy of allergic disease has long constituted injection therapy using crude extracts of the offending allergen to effect the formation of blocking IgG or IgA antibodies that can combine with the allergen before it reaches the target cells sensitized with IgE of the same specificity. Newer approaches to immunotherapy are now being developed. Depression of IgE formation with a concomitant clinical improvement can be observed in patients allergic to Timothy grass pollen after treatment with a low molecular weight fraction of the pollen extract, antigen D, which possesses the properties of a hapten. As hapten, this molecule is not immunogenic, and at the same time it reduces IgE formation, probably by a feedback inhibition process. This observation holds promise for the treatment of some common forms of allergy by using haptens derived from the offending allergen.

A different form of immunotherapy involves interference with the bridging mechanisms known to be a necessary antecedent to histamine release from target cells. Cell-bound IgE must bridge to effect a target cell membrane distortion, which is necessary for mediator release. This bridging seems to be accomplished by the combination of multivalent antigen with the divalent IgE. It has been possible to achieve successful desensitization of

some penicillin-allergic patients with a univalent penicillin derivative, benzylpenicilloyl-formyl lysine, so that these patients could tolerate further administration of penicillin. Low molecular weight components of ragweed allergens, which are not skin active in individuals allergic to ragweed extract, possess the properties of haptens capable of binding to the combining site of IgE, probably in a univalent fashion. This process thus blocks the combining of these cell-bound IgE molecules with polyvalent allergen and obviates the bridging phenomenon and subsequent triggering of mediator release. Other promising work has involved the synthesis of extremely low molecular weight peptides, whose amino acid composition mimics the terminal part of the Fc portion of IgE. Administration of these peptides competes with IgE for the receptor sites for IgE on the target cells and ostensibly prevents the optimal binding of IgE to the basophil or mast cell.

The frequent demonstration of IgE antiparasite antibodies in animals infested with various parasites has led to speculation concerning the possible beneficial role of such antibodies in host defense. The laboratory rat is easily infested with the nematode *Nippostrongylus brasiliensis.* By 14 to 16 days after infestation with the larvae, the animals undergo dramatic self-cure manifested by a sharp drop in nematode egg production and by expulsion of the mature worms from the intestine. There is evidence that mast cells are involved in the host response to *N. brasiliensis,* because soon after the adult worms reach the intestine, there is degranulation and disappearance of the mast cells from the lamina propria. Repeated administration of an antiserum primarily directed against the mast cells prolongs the infestation and increases the number of eggs produced by adult worms in the gut. Administration of an antihistamine reduces worm loss from the intestine. In infested rats, active cutaneous anaphylactic responses can be elicited with worm extracts as early as day 10. At present, it is tempting to suggest that the IgE antibody-mast cell system plays a role in host defense to parasitism, although this hypothesis awaits sound definition in humans.

The Complement System

The term complement denotes a system of normal human serum factors that are activated by an antigen-antibody complex and subsequently mediate several important immunologic functions (Table 4). The factors of the classic pathway have been designated with numbers (C1 to C9), and their typical reaction cascade proceeds in the following order: C1, C4, C2, C3, C5, C6, C7, C8, and C9. When components acquire an enzymatic or other biologic activity, the accepted nomenclature indicates this new function by inserting a bar over the number of the component.

Table 4. Functions of the Complement System

Amplification mechanisms for antigen-antibody reactions.
Generation of chemotactic factors (C3a, C5a).
Generation of anaphylatoxins (C3a, C5a).
Generation of opsonic activity (C3b).
Immune adherence of foreign invaders to neutrophils/macrophages (C3).
Generation of kinin-like substances (C2).

For many years, the lysis of sheep red blood cells by antibody and complement served as the model of complement activity. However, further study has defined many more

functions of the complement system in addition to lysis of foreign cellular material.

Initially in the complement cascade, C1 binds to an antigen-antibody complex somewhere along the Fc portion of the antibody. One molecule of C1 binds to one molecule of IgM, but two molecules of IgG are required for the binding of one molecule of C1. C1 is a macromolecular complex with a molecular weight of about 1 x 10^6 daltons. It can be dissociated into three distinct protein subunits, C1q, C1r, and C1s. C1q carries the binding site for antibody. C1q probably activates C1r, and C1r can act on C1s proteolytically to active this last subunit. C1s carries the proenzyme site that on activation is converted to C1, the enzyme that subsequently acts on C4 and C2. C1 cleaves C4 and C2, forming C42, an active enzyme known as C3 convertase. An important amplification occurs here in that a single C1 molecule can cleave many C4 and C2 molecules.

C42 cleaves C3 into C3a and C3b, with additional amplification here also in that many C3 molecules can be cleaved by one C42 complex. C3a splits off, and C3b becomes attached to the cell membrane. C5, C6, and C7 are then bound, forming the so-called heat stable intermediate. After C7 is bound, the final product is a trimolecular complex C567 that is also capable of attaching to cell membranes. This complex has a relatively long half-life (30 seconds) and allows the binding of the complex to sites somewhat distant from the initial complement fixation site so that the action of complement components may be allowed to spread to contiguous sites where possibly needed. C8 and C9 are then required for lysis. In addition to binding at the complement-fixing site, C8 binds to C567. C9 then binds to C8. C8 is believed to play the major role in lysis, with C9 probably acting as cofactor. Even after the binding of C8 and C9, lysis can be prevented by certain experimental manipulations, suggesting that other undefined steps complete the process of lysis.

The morphologic correlates of complement action are lesions on the cell membrane. These lesions are circular and measure about 100 Å in diameter, but whether they are true holes is uncertain. Cells can show that a multiplicity of C567 sites were produced. The lipid portion of the membrane is primarily involved in the lesion.

Homeostatic control of complement fixation occurs. Although C1 inhibitor cannot stop the ongoing complement activation, it is instrumental in preventing the autoactivation of C1. The short half-life of C3 convertase (C42) limits its duration of activity at the complement-fixing site. Interestingly, the C3 convertase generated by cobra venom factor has a half-life long enough (20 hours) to cause the total consumption of complement in vivo. A C3b inhibitor exists to control C3 activation.

An alternate pathway of complement activation exists that bypasses the early components, C1, C4, and C2, and begins with C3. It is mediated by the properdin system requiring properdin, a euglobulin of 230.000 daltons molecular weight, and at least one other factor. It appears that IgA and possibly IgE, which do not fix C1, are capable of activating complement via this alternate pathway.

The biologic actions of complement go far beyond lysis of foreign cellular invaders. Both neutrophils and macrophages adhere to bound C3, and these immune adherence reactions are involved in the first stages of phagocytosis, probably facilitating ingestion. A kinin-like fragment is formed from C2 during the activation of C42 that has the capacity to increase

vascular permeability. C3a, produced by the action of C42, acts as an anaphylatoxin and as a chemotactic factor, as does C5a, which is believed to be the more potent chemotactic factor. The complement and clotting systems are intimately interrelated, and complement activation enhances platelet procoagulant activity.

Hereditary angioneurotic edema is a disorder of the complement system transmitted as an autosomal dominant trait. Clinically, it is marked by paroxysms of swelling of subcutaneous tissues, the gastrointestinal tract, or the upper respiratory tract, sometimes with fatal laryngeal edema. The genetically determined error involves C1 inhibitor, either in its absence or through the biosynthesis of a nonfunctional form. Specifically, the inhibitor involved is C1 esterase (C1s) inhibitor, which has been implicated because of the following findings. Highly purified C1s produces angioedema when injected in human skin. Experimental animals deficient in C4 are much less responsive to an injection of C1s than are normal animals. C2-deficient humans are poorly responsive to an injection of C1s. Injection of C1s into patients with hereditary angioneurotic edema can provoke an attack. The plasma of patients with hereditary angioneurotic edema contains measurable levels of C1s, unlike the plasma of normal individuals. Liver biopsies of these patients do not show C1s inhibitor activity by immunofluorescent techniques, whereas normal hepatic parenchymal cell will, because this is the site of synthesis of C1s inhibitor. It appears that the offending mediator that is liberated during this autoactivation of C1 and the noninhibition of C1 is the C2 kinin, although the mechanism prompting the activation of C1 in these patients is not yet known.

A number of individuals have been identified who lack C2. These individuals do not seem to have an increased propensity to infection, and any direct pathologic consequences of C2 deficiency have yet to be described, although syndromes resembling systemic lupus erythematosus (SLE) have been reported. A congenital hypercatabolism of C3 is a well defined though extremely rare syndrome, resulting in an inordinate susceptibility to pyogenic infections. A C5 dysfunction is also known in which C5 generates little lytic activity although total serum C5 concentration is normal.

In most inflammatory conditions, serum complement levels are increased during the acute phase. However, with certain conditions hypocomplementemia results. These conditions most often involve immune complexes that consequently fix complement and thus result in hypocomplementemia. SLE is notable in this regard, with immune complexes deposited in renal or vascular tissue. Evidence suggests that the lesions in tissues of patients with SLE are not due to cytotoxic activity of autoantibodies but rather to the physical deposition of immune complexes of autoantibodies and the respective antigens. Correlating with this notion is that decreases in serum complement levels and fluctuations of deoxyribonucleic acid (DNA) antibody levels occur with clinical exacerbations. Immune complex is also found in patients with cancer. In a few of these patients, the immune complex disease is manifested by a clinical nephrotic syndrome due to the glomerular deposition of complexes of tumor antigen and tumor antibody. In most, evidence of immune complex disease is gained indirectly by the demonstration of decreased levels of serum C1q.

Dengue is an acute viral disease caused by an arbor B virus, which is endemic in tropical areas. One of its manifestations is increased vascular permeability combined with intravascular coagulation. Since the complement system can generate biologic activities causing increased vascular permeability, chemotaxis of phagocytes, histamine release from

cellular elements, and enhancement of platelet procoagulant activity, studies have attempted to characterize complement activity during the course of dengue hemorrhagic fever. There exists an inverse relationship between serum complement levels and the severity of the illness, with both the classic and alternate complement pathways apparently being activated. These patients show increased catabolic rates of C3 and C1q, especially during the period of hemorrhagic shock. The implication is strong that complement activation is a major factor in the development of the shock syndrome in dengue fever, although the inciting event for complement activation is unknown. Since both pathways are activated, a multiplicity of inciting substances could be suggested, notably dengue virus particles or endotoxins. Interestingly, immune complexes may also be suggested. Since four different cross-reacting serotypes of the dengue virus are known, reinfection of an individual with a different serotype could result in the anamnestic production of antibodies that could not neutralize the virus. The virus could continue to replicate and combine with non-neutralizing antibodies, resulting in accumulation of immune complexes capable of activating the complement system. The acute vascular disorders seen in some bacterial infections and other viral infections may involve a similar pathobiologic mechanisms involving complement activation in some unknown manner.

The Clotting System

Blood coagulation begins with the activation of Hageman's factor (factor XII). Aside from this role, Hageman's factor can initiate several different pathways relevant to inflammation. It was originally described as that factor that was activated upon the contact of fresh blood with negatively charged glass. Now it is apparent that activated Hageman's factor also can operate with other cofactors to produce plasmin from plasminogen. Plasmin has the capacity to activate C1 and C1- and to cleave C3 to yield the anaphylatoxin and chemotactic factor C3a. Another pathway involving Hageman's factor is the kinin system. Activated Hageman's factor and Hageman's factor fragments can convert prekallikrein to kallikrein, the enzyme that cleaves bradykinin from kininogen. As a consequence of kinin generation, a fragment (Kf) appears that promotes the combination of C1- with C4 and C2.

Lymphokines

This term denotes certain biologically active substances elaborated by sensitized lymphocytes, presumably T-cells, in the presence of the specific antigen. Lymphokines constitute an important amplification system, since a single sensitized lymphocyte can augment the magnitude of a cellular immune response by a factor of several thousand by the elaboration of the molecules.

Migration Inhibition Factor (MIF)

This is probably one the best known lymphokines and was earlier described from experiments that demonstrated the inhibition of wandering immune cells of animals sensitive to the tubercle bacillus but not of those cells from nonsensitive animals. Further tests of specificity showed that immune cells sensitive to ovalbumin were inhibited from migrating in the presence of ovalbumin but not in the presence of diphtheria toxoid. Humans who are sensitized to *Brucella* possess immune cells whose migration is inhibited in the presence of *Brucella*, but this is not the case for nonsensitized individuals. This in vitro phenomenon is not only antigen specific but also much additional work has established the migration

inhibition assay as a test of delayed hypersensitivity or cellular immunity.

The primary cell whose migration is inhibited is the macrophage. MIF appears to be synthesized by sensitized lymphocytes. It has been shown that purified lymphocytes from PPD-sensitive animals can produce MIF on exposure to PPD and that macrophages either from PPD-sensitized animals or from animals never exposed to PPD are both inhibited. Thus, the lymphocytes is the antigen-sensitive cell that produces MIF on an encounter with a specific antigen, but the action of MIF on macrophages is a secondary nonspecific effect. The number of lymphocytes necessary to produce a measurable effect is exceptionally small. The in vivo correlate of this stoichiometry is that at the site of a delayed hypersensitivity reaction only a small percentage of the accumulated cells are sensitized lymphocytes, and the remainder constitute a large population of cells, mostly macrophages, whose migration has been inhibited at the site of the response.

Human MIF is a molecule weighing about 23.000 daltons. The protein nature of MIF is indicated by the ability of chymotrypsin to completely destroy its activity. MIF can be synthesized by sensitized lymphocytes within 6 hours after interaction with the specific antigen. The effect of MIF on macrophages is not only to immobilize them at inflammatory sites where they are needed but also to activate them to a state of increased pinocytic activity and increased size of activated macrophages are believed to be important to the cells in the defense against fungi, viruses, and bacteria and in the immune response to allografts and tumors. A macrophage-activating factor has been reported, but it appears indistinguishable from MIF.

Chemotactic Factor

As already discussed, the serum complement system generates chemotactic factors C3a and C5a because complement components are cleaved during the cascade. Sensitized lymphocytes also produce a chemotactic factor for macrophages in an antigen-specific manner similar to that for MIF production. This factor is distinct from MIF, and the combined effects of these two factors can be conceptualized as attracting macrophages to inflammatory sites and inhibiting their migration elsewhere. This factor can be produced artificially by stimulating lymphocytes with the nonspecific mitogen concanavalin A. The supernatants from such stimulated cultures contain the factor, sometimes called lymphocyte-derived chemotactic factor (LDCF). Patients with carcinoma show defects of the chemotactic response of their macrophages to LDCF that are more pronounced with more advanced stages of disease. Tumor removal or immunotherapy with BCG can enhance the chemotactic response. This impaired chemotactic response could be related to impaired tumor immunity and could result from an inability to respond appropriately to LDCF rather than inappropriate production of LDCF, because the production of LDCF can be normal in patients with Hodgkin's disease. In contradistinction, a patient with chronic mucocutaneous candidiasis, a disease also associated with defects of cell-mediated immunity, showed both impaired chemotaxis to LDCF and to C5a and failure of LDCF production on exposure to *Candida* antigen. Thus, in diseases with deficient chemotactic responses of deficient production of chemotactic factors, the immune system may not be able to localize cells in the area of a foreign challenge.

Lymphocytes produce a factor chemotactic for neutrophils. Physicochemically, this factor is distinct from MIF and from the chemotactic factor for macrophages. Lymphocytes

can also produce a distinct factor that is chemotactic for eosinophils, the production being dependent on the presence of antigen-antibody complexes with the requirement that the antigen part of the complex is identical to the antigen for which the lymphocytes are sensitive. It has been suggested that this factor may play a role in autoimmune diseases that have a component of cell-mediated immunity. Certain collagen diseases demonstrate eosinophilia, and experimental autoimmune thyroiditis becomes manifest as thyroid lesions with infiltration of eosinophils.

Cytotoxic and Cytostatic Factors

A soluble protein factor, distinguishable from MIF, can be produced by sensitized rat lymphocytes stimulated with specific antigen that could kill normal rat fibroblasts. Lymphotoxin can also be obtained from human adenoid lymphocytes. Sensitized human lymphocytes can also produce a factor, called cloning inhibitory factor, that can reduce the growth of tissue culture cells but does not kill them. The biologic relevance of lymphotoxin and cloning inhibitory factor remains to be established, although they could presumably be active against tumor cells and transplanted tissue.

Blastogenic Factor

Stimulation of sensitized lymphocytes can result in the production of a factor with mitogenic properties, ie, a factor that induces lymphocyte division. It is possible that the production of such a factor by a small number of sensitized lymphocytes can recruit nonsensitive bystander lymphocytes, enlarge their numbers, and thus expand the population of responding immune cells and subsequently augment the production of mediators.

Interferon

Interferon is a substance with powerful antiviral properties, among others. It is produced by lymphocytes and by a variety of other cells in response to viruses and other inducers such as nucleic acids, bacterial products, and synthetic polymers. Interferon tends to be most active in the same species that produced it. This substance does not act extracellularly to decrease viral attachment or penetration into a cell but acts intracellularly to limit viral synthesis. The capacity to produce interferon is not a property of the viral genome but resides within the genome of the host cell. Interferon also appears to possess some antitumor properties.

Secretory Products of Macrophages

Recent research has made it clear that many substances are secreted by the macrophage into the immediate environment. The exact nature of most of these substances is unknown, but a number of them influence the behavior of neighboring cells. An inhibitor of cellular proliferation, having a molecular weight of 1400 daltons or less, is synthesized and secreted by macrophages. It is cytostatic without being cytotoxic. It can inhibit the proliferation of lymphocytes and can decrease the in vitro production of antibody, probably by inhibiting the expansion of B-cell numbers. Also, macrophages produce a molecule that stimulates T-lymphocyte proliferation. This molecule also enhances antibody production by B-cells. The exact biologic significance of these molecules is yet unknown. The inhibitory

molecule possesses the capacity of also inhibiting tumor cell growth in vitro, and so some researchers wish to ascribe to this molecule a role in effector immunity. Equally tenable is that the inhibitory factor may be a regulator of afferent immunity, since it could theoretically inhibit the production of increased numbers of lymphocytes. The same could be true for the amplifying effect of the stimulatory molecule. As such, the macrophage may be a likely candidate for a crucial cell-modulating afferent immunity in a homeostatic fashion in addition too its known activity as phagocyte in efferent immune processes.

Molecules of Innate Immunity

In its broadest definition, innate immunity includes nonspecific immunologic functions. The idea of specificity is the foundation of immunology. Immune responses are defensive responses to foreign invaders, and, without the immune system's capacity to discriminate self from nonself or similar from foreign, immune attacks would be mounted randomly and without purpose. Therefore, the description of innate, nonspecific defensive functions was once considered so heretical that this attitude resulted in the martyrdom of a young researcher, Louis Pillemer.

Properdin

Pillemer described a serum component, properdin, that reacted with a constituent of yeast to activate C3, bypassing C1, 4, and 2 (hence, the alternate pathway) and thus seeming to have significant antimicrobial activity. But properdin was not an antibody, and therefore this system lacked specificity. Pillemer's work was ridiculed, his papers were refused publication, his peers rebuked him, and his ideas were ignored. The damaging effects of unenlightened and relentless criticism prevailed, and this young researcher returned to his laboratory one evening, drank barbital buffer, and committed suicide.

It is now well established that antimicrobial activity in the serum can be mediated either by properdin-complement or by antibody-complement systems. In 1968, properdin was isolated and was determined to be like a beta-globulin and distinct from immunoglobulins or complement components with a molecular weight of about 223.000 daltons. It has proved difficult, however, to define the factors required along with properdin to activate the alternate pathway, but at least thee other factors appear necessary. Whether antibody or antibody-like factors with their inherent specificity are required remains to be decided. Nevertheless, the properdin system is responsible for significant antibacterial and antiviral activities.

Opsonins

The complement system, chiefly through C3a and other uncharacterized molecules, plays a significant role in the opsonization of bacteria for the facilitation of phagocytosis. The increased susceptibility of neonates to gram-negative infections was once believed to be purely due to the physiologic and transient deficiency of IgM antibodies, since antibodies of this class play a major role in defense against such organisms and are also remarkably efficient opsonins. However, this is only a partial explanation, for the addition of purified IgM to neonatal serum fails to significantly enhance its opsonic activity. A neonatal deficiency of other types of opsonins must be postulated.

Interferon

This molecule has already been mentioned. It is an innate defense mechanism so primitive that it is found in teleost fishes.

Lysozyme

Lysozyme has undisputed bactericidal activity because of its ability to hydrolyze cell wall mucopolysaccharides chiefly of gram-negative bacteria. Lysozyme is present in serum, in cells (neutrophils and macrophages), and in external secretions such as nasal mucus, saliva, and tears. Its antibacterial spectrum is narrow, but it bears a biochemical specificity for a certain linkage of chemical groups.

Natural Antibodies

Although an antibody by definition denotes specificity, natural antibodies are worthy of mention here because they seem to appear in the body without demonstrable prior antigenic stimulation. The best known natural antibodies are isohemagglutinins such as anti-A and anti-B present in the sera of persons lacking the corresponding antigen on their erythrocytes. However, a wide variety of other natural antibodies have been identified with reactivities against bacteria, viruses, bacteriophages, and protozoa. It is not unlikely that these natural antibodies actually could result from an encounter with an unrelated antigen that bears a specificity that cross-reacts with the specificity against which the natural antibody is directed. Substances with blood group A reactivity can be found in horse saliva, porcine gastric mucosa, house dust, and in certain pneumococci. Contact with any of these antigens could result in the production of anti-A isohemagglutinin, even if there were never any contact with human red cells of blood group A. Nevertheless, some contend that natural antibodies are genetically determined proteins that are manufactured independent of antigenic stimulation. The presence of agglutinins in invertebrates, which lack lymphoid tissue, could support this view.

Immunogenetics

The nature of the major histocompatibility or transplantation antigens is determined by a system of genes on chromosome 6 known as the human leukocyte antigen (HLA) complex or, sometimes, MHC. A system of genes, by genetic definition, is composed of two or more linked loci showing less than 1 per cent recombination and a nonrandom combination of the alleles of the loci (ie, genetic disequilibrium). Estimates of the number of genes in the HLA complex vary from 200 to as many as 2000. The genes of the HLA complex are known to code for histocompatibility antigens on the surfaces of all nucleated cells. These histocompatibility antigens represent the strongest antigenic barrier to successful organ transplantation.

Two of these genes, the A and B genes, code for the structure of cell-surface glycoproteins. Pedigree data have shown that the A and B loci (formerly called the LA and FOUR loci, respectively) are closely linked. Each of these loci has a separate set of alleles that seem to be codominant. Each allele is expressed as a different HLA antigen on the cell surface. As of this writing, there are 19 well-recognized alleles for the A locus and 26 for the

B locus. Haplotypes are pairs of alleles, one from the A locus and one from the B locus, on the same chromosome. Since the A and B loci are very closely linked, the pair of genes will be inherited by an offspring unless a rare recombinational event occurs. Since the offspring will inherit one chromosome (haplotype) from each parent, the full expression of the A and B loci will consist of four surface HLA antigens unless the individual is homozygous at the A or B locus or if the individual expresses an unrecognized HLA antigen.

A major function of the HLA-A and HLA-B antigens is the expression of self. These antigens elicit very strong cytotoxic reactions from immunocompetent cells of different HLA phenotypes and thus are targets present on histoincompatible transplanted tissue. They are probably the major antigens eliciting graft rejection. Tissue typing is performed on peripheral lymphocytes because they are easily available source of nucleated cells, the assumption being that the types and density of HLA antigens detected on the surface of lymphocytes will be characteristic of the organ to be transplanted. Antisera for typing are obtained from multiparous women, recipients of multiple blood transfusions, recipients of rejected kidney transplants, or from volunteers sensitized by an injection of leukocytes or by a skin graft. All such individuals have had the opportunity to become sensitized and mount an antibody response to foreign tissue alloantigens.

If tissue is transplanted between identical twins, no rejection occurs. Skin grafted between HLA-identical siblings shows a long survival time. When donor and recipient siblings share only one haplotype (haploidentical), skin survival is intermediate; and when donor and recipient siblings are totally histoincompatible, survival time is the shortest. In kidney transplantation, the ideal donor is a sibling who is HLA identical and ABO compatible. In this instance, transplant acceptance is optimal, and the amount and intensity of immunosuppressive therapy needed are minimal.

When one examines the fate of tissue transplanted between unrelated individuals, the correlation with HLA matching is less clear. It was shown earlier that the survival time of skin grafts between HLA-identical unrelated individuals was very similar to that between randomly chosen pairs of individuals. Likewise for kidney transplantation, graft survival may be good despite several incompatibilities.

It soon became clear that typing for the serologically defined A and B specificities was not a sufficient analysis for transplantation and that perhaps other antigens specified by loci other than A or B in the HLA complex participated in transplantation immunity. To address this possibility, advantage was taken of earlier observations that lymphocytes from different individuals would undergo blast transformation and mitosis when mixed together in culture. Further study showed that such an MLC was a measure of disparity of histocompatibility antigens between the two individuals. The stronger the reaction, the more the antigenic disparity. The MLC measures disparity for presumably all histocompatibility antigens involved. That the MLC does indeed measure disparity other than that of the A and B loci was elegantly confirmed by sibling experiments demonstrating that antigens of HLA-A and HLA-B mismatched siblings could be mutually nonstimulatory in MLC, whereas antigens of other pairs of HLA-A and HLA-B identical siblings could be strongly reactive in MLC. Another locus in the HLA complex, the D locus, has been postulated to govern MLC reactivity. This locus is nearer to the B locus, and its alleles can be in genetic disequilibrium with certain B locus alleles. The biochemical products of the D locus cannot be detected

serologically but only by appropriately adapted MLC techniques involving lymphocyte mitosis.

Therefore, successful allotransplantation must take into account not only the serologically defined (SD) antigens of the A and B loci but also the lymphocyte defined (LD) antigens of the D locus. In a large series of cadaveric renal transplants, kidney survival clearly depended on the degree of matching at the A and B loci. However, further analysis revealed the influence of the D locus. First, when both B antigens were shared by donor and recipient, kidney survival was better than when just one was shared. As previously mentioned, the alleles of the B and D loci can be in linkage disequilibrium, and complete matching at the B locus could sometimes result in inadvertent matching at the D locus. Second, the best graft survival was seen in cases in which donor and recipient had poorly responsive MLC reactions, ie, in which the greatest compatibility at the D locus was obvious, in addition to good matching at the A and B loci. Although bone marrow transplantation is a much more immunologically complex phenomenon than is solid organ transplantation, experience with this therapeutic modality also underscores the importance of MLC matching and graft survival. Patients with aplastic anemia who are treated by bone marrow transplantation show the best bone marrow graft survival when MLC reactions between donor and recipient are weak. A successful bone marrow transplantation from an HLA-, MLC-identical unrelated individual has been carried out for chronic granulomatous disease.

There are other genes in the complex. Evidence from analysis of the murine homologue of the HLA complex, the H-2 system, reveals that an I region exists that contains immune response (Ir) genes. Ir genes govern the ability of an animal to make an immune response to certain viruses and antigens. How this occurs in molecular terms is unknown, but this system represents another level of immune recognition in addition to immunoglobulin receptors on B-lymphocytes and the receptors on T-lymphocytes. Some believe that the human D locus may be the counterpart of the murine I region. Therefore, reactivity in MLC may not necessarily be a measure of genetic disparity at the D locus but rather a measure of the efficiency governed by the D locus to mount an immune response to transplantation antigens of the A, B, and other loci.

Disease Associations and HLA Antigens

Associations between diseases and certain HLA antigens are beginning to emerge (Table 5). It is interesting that murine resistance to the Gross leukemia virus is determined in great part by the H-2 phenotype of the mouse. There is also evidence that the H-2 complex is involved in the susceptibility to other leukemogenic viruses, the Friend virus and the Tennant virus. In addition, the existence of Ir genes closely linked to the genes that code for surface histocompatibility antigens lays the foundation for investigating the relationship of the human HLA-A and HLA-B antigens to disease.

There can be certain errors involved in searching for an association of a disease with a certain HLA antigen. Sampling errors may occur when studying racially or culturally skewed populations, for there are wide variations in the frequencies of particular HLA antigens in various races. The A locus antigen A1 and the B locus antigen B8 are very common in European populations, whereas these are very rare or completely absent in Malays. A9 and BW15 are common in Malays, but rare in Europeans. It appears that A1, A3,

B8, and B14 are lacking in Japanese. Thus, spurious relationships between a disease and an increased or decreased frequency of a certain HLA antigen may be generated if such ethnic and geographic considerations are not taken into account. Thus, blacks with lupus erythematosus show an increased frequency of BW35, whereas whites with this disease show an increased frequency of BW15. Such a finding does not really clarify the relationship of BW35 and BW15 to lupus erythematosus. Other sampling errors result from the study of diseases that are actually heterogeneous. An increased frequency of B7 has been found in females with Hodgkin's disease of the nodular sclerosing type only, whereas this is not the case for all histologic types of Hodgkin's disease. An increased frequency of BW35 is found with retinoblastoma of the hereditary type only. Therefore, true associations may not be appreciated unless the heterogeneity of the disease under study is considered.

Table 5. Disease Associations With B and D Locus Antigens

B5	B13
Behçet's disease	Severe streptococcal infections
Malignant melanoma	Psoriasis
B7	Pemphigus
Pernicious anemia	B27
Multiple sclerosis	Postinfection arthritis
Paralytic poliomyelitis	Rheumatoid arthritis
Ragweed allergy	Ankylosing spondylitis
Breast carcinoma	Reiter's syndrome
B8	Frozen shoulder
Gluten-sensitive enteropathy	Acute and chronic uveitis
Dermatitis herpetiformis	Ragweed allergy
Chronic active hepatitis	BW35
Myasthenia gravis	Infectious mononucleosis
Diabetes mellitus	Retinoblastoma
Graves' disease	Sin 2
Addison's disease	Nasopharyngeal carcinoma in Singapore Chinese
Thyroiditis	DW2
Asthma	Multiple sclerosis
Ragweed allergy	DW3
Systemic lupus erythematosus	Addison's disease
Hodgkin's disease	Diabetes mellitus
Acute myelocytic leukemia	Celiac disease
B12	Sjögren's syndrome
Atopic symptoms in childhood nephrotic syndrome	DW4
Retinoblastoma	Adult onset rheumatoid arthritis.
Chronic myelocytic leukemia	
Chronic lymphocytic leukemia	
Non-Hodgkin's lymphomas	

Typing errors can also result. Typing antisera are often multispecific, detecting not just one HLA antigen of the A or B loci. Therefore, a large panel of antisera must be used. Antisera can also cross-react, such as the cross-reacting ability of anti-A3 with A11. Disease states and the ingestion of certain drugs may alter the surface antigenic display of lymphocytes, since HLA antigen turnover is rapid, with constant surface shedding and new production. Serious statistical errors are also operative. Given the relatively large number of HLA antigens, it has been estimated that a relationship between a disease and a certain HLA antigen could be derived by chance alone approximately once in every 100 examinations. Therefore, if one accepts the 5 per cent level as statistically significant, a correction factor must be included to account for the number of HLA specificities being examined. If 20 specificities are examined, the 5 per cent level is reduced to 0.25 per cent for statistical significance.

With critical analyses, significant disease associations are now known. It seems that the great majority of these associations are with antigens specified by the B locus. For infectious diseases, most patients who develop postinfection arthritis after infections with *Salmonella, Shigella,* or *Yersinia* are B27 positive, although other genetic factors seem involved. For neoplastic diseases, there is a suggestion that B12 may show an increased frequency in cases of non-Hodgkin's lymphomas and that B5 may be associated with increased survival in patients with bronchogenic carcinoma. An association between nasopharyngeal carcinoma in Singapore Chinese patients and the HLA antigen Singapore 2 (Sin 2) seems confirmed.

Some of the clearest associations with HLA antigens are emerging from studies of patients with immunopathic diseases. An increased frequency of B8 is found in chronic active hepatitis, systemic lupus erythematosus, and myasthenia gravis. Studies of multiple sclerosis show increased frequencies of B7. In ragweed hypersensitivity, susceptibility seems to be associated with B27, and some studies show a relationship with the A locus antigen A7. Atopic symptoms, particularly hay fever, are more common in children with the nephrotic syndrome who possess B12. The different clinical manifestations of atopy seem especially related to the haplotype A1/B8.

We are now forced to understand why there should be an association between surface HLA antigens that are the targets of tissue rejection and diseases of diverse etiologies. Numerous explanations are extant. It could be possible that a surface HLA antigen could act as a receptor for a virus, facilitating cell penetration and thus predisposing individuals with that antigen to diseases caused by that virus. The explanation of molecular mimicry has also been evoked. It is known that a cross-reactivity exists between certain HLA antigens and streptococcal M protein and that certain H-2 antisera can neutralize a murine leukemia virus. Therefore, it could be possible that some microbial antigens could be so similar to cell-surface HLA antigens that an effective immune response would not be mounted, owing to the interpretation of the microbe as self.

A very likely explanation is that the HLA antigens of the A and B loci are closely linked to immune response genes, and a B locus antigen may in fact be a marker for a particular nearby Ir gene. Since the D locus is probably involved with immune responses, one would speculate that D locus typing would be more sensitive in revealing disease associations. In fact, this appears to be true. For multiple sclerosis, myasthenia gravis, and rheumatoid

arthritis, the association with a particular D locus allele is stronger than the previously described associations with B locus alleles. Similarly, celiac disease is strongly associated with the D locus determinant DW3 and only secondarily with B8, and a linkage disequilibrium between BW3 and B8 is postulated. Patients with Sjögren's syndrome and rheumatoid arthritis do not show such an association, and this latter clinical entity could thus be considered genetically distinct from Sjögren's syndrome alone.

The questions arise as to why all individuals with a particular disease do not possess the same HLA phenotype and why all individuals with a given phenotype do not demonstrate a certain disease. Since the human population is exceptionally outbred, many crossovers must have occurred between the B and D loci, and therefore a certain Ir gene may not always be found with a certain B locus allele. A new gene system has recently been proposed with the identification of antigens present on the surface of B-lymphocytes but not T-lymphocytes. These B-lymphocyte alloantigens appear distinct from the A, B, and D loci but are believed to be coded for by gene loci on chromosome 6 and close to and in linkage disequilibrium with the A-D genes. Patients with multiple sclerosis show an inordinate incidence of a particular B-lymphocyte antigen. In addition, asthma, gluten-sensitive enteropathy, dermatitis herpetiformis, and chronic mesangiocapillary glomerulonephritis have all been associated with certain other of these antigens. Some believe that the B-lymphocyte alloantigens may actually be the cell-surface end-products coded for by genes at the HLA-D locus. However, if the B-lymphocyte alloantigens do indeed prove to be a new and distinct gene system, typing for these and for the antigens of the A-D loci could give a more complete description of an individual's phenotype and possibly thus lead to firmer disease associations. Also, the genetic influence on many diseases is clearly polygenic, and thus susceptibility to disease can be only partially related to HLA phenotype in certain cases. For instance, it has been estimated that HLA contributes only one-thousandth of the causative factors to the genesis of Hodgkin's disease.

Cancer Immunology

Enthusiasm for the notion that the growth of malignant cells is regulated by an immunologic mechanism has waxed and waned over the years. To entertain an essential relationship between immunity and malignancy, a number of crucial issues must be considered. The first of these is that tumors displays antigens.

Tumor Antigens

It was first shown that sarcomas induced in inbred mice by chemical carcinogens demonstrated new antigens specific for the tumor. Another major discovery was that certain DNA viruses could produce tumors in experimental animals and that animals immunized with the oncogenic virus could reject tumors induced by that virus, thus indicating that the tumor cells contained new antigens that were in some way characteristic of the inciting virus. Serologic techniques have been employed with great incisiveness, so that in every animal tumor system that has been appropriately studied, tumor-associated antigens have been demonstrated.

Burkitt's lymphoma was the first human tumor studied serologically, and at least five distinct antigens are associated with this cancer. Three of these seem to be characteristic of

the Epstein-Barr virus (EBV), and one seems to be characteristic of the family of herpesviruses. Therefore, searches for human tumor antigens may have an extra benefit, for they may give some clue as to the etiologic agent. Most human tumors that have been rigorously studied appear to posses new antigens foreign to the host but related to the tumor. Human tumors that are demonstrably antigenic include melanoma, neuroblastoma, glioma, uroepithelial carcinoma, ovarian carcinoma, sarcoma, and colon carcinoma.

For epidermoid carcinomas of the head and neck, little progress has been made in demonstrating or identifying antigens peculiar to this malignancy. However, lymphocytes from patients with head and neck epidermoid cancer can react with a tumor cell line derived from a laryngeal epidermoid carcinoma. This suggests that perhaps all head and neck epidermoid cancers share new antigens, but their nature remains undefined.

Nasopharyngeal carcinoma (NPC) is an uncommon tumor somewhat restricted to one ethnic group, the Southern Chinese. Experimentally, explants of this tumor give rise to lymphoblastoid cell lines showing the presence of antigens specified by EBV. The original suspicion that EBV was somehow related to NPC was derived from serologic studies demonstrating that virtually 100 per cent of NPC patients had detectable antibody in high titer to the capsid antigen of EBV. Furthermore, the titer of these antibodies falls dramatically in those patients who go on to become long-term survivors of the disease. Additional evidence supporting the association of EBV with NPC came from hybridization experiments documenting the significant annealing of EBV-specific complementary RNA (cRNA) with DNA from NPC biopsy specimens and the demonstration of in situ hybridization of EBV-cRNA with DNA epithelial cells of the tumor. These last data indicate that EBV genomes are present in the tumor cells, not only in the lymphocytes, some of which may be only infiltrating cells, but also in the epithelial cells. This association of EBV antigens with NPC is not sufficient to establish causality. EBV may merely be an associated passenger virus, or EBV could be one of a necessary constellation of etiologic factors working perhaps in conjunction with Sin 2.

An etiologic association, although weak, has been preferred for the herpes simplex type 1 virus (HSV-1) and head and neck epidermoid cancer. The first report claimed that sera of patients with advanced cancer of the lip, oral cavity, and oropharynx have positive complement fixation reactions with nonvirion (ie, nonstructural) antigens of HSV-1, whereas sera from patients without cancer did not. In a more extended study of 94 patients with epidermoid cancer of the oropharynx or larynx, the majority of these patients, including those clinically cured, showed positive complement fixation reactions with nonvirion HSV-1 antigens. In addition, an argument has been put forward for the participation of HSV-1 in the carcinomatous transformation of leukoplakia, based on the finding that a specific increase of cell-mediated immunity to HSV-1 could be observed in some cases of leukoplakia. Antigens specified by HSV-1, however, have not been demonstrated in the nucleus or cytoplasm or on the surface of epidermoid tumor cells.

Colon carcinoma is worthy of note here because of the numerous studies that have afforded insights into the specificity and nature of some tumor-associated antigens. The Hellström colony inhibition assay is used for the detection of immunity against tumor antigens and is predicated upon the killing or growth inhibition of tumor cells by specificity immune lymphocytes. By comparing the number of colonies of tumor cells formed in a tissue culture

vessel when immune lymphocytes are admixed, the percentage of tumor cells killed or inhibited by the immune lymphocytes can be determined, and the demonstration of tumor antigenicity can be appreciated. Employing this assay, it has been shown that lymphocytes from patients with colon carcinoma are reactive against autochthonous and allogeneic colon tumor cells, indicating that colon carcinomas may possess a common antigen. Lymphocytes from patients with breast or lung carcinomas, malignant melanomas, or sarcomas were not reactive against colon carcinoma cells, and lymphocytes from patients with colon cancer were not inhibitory for a variety of other neoplasms, implying that the antigen was peculiar to colon cancer. The suspicion that this antigen was a fetal antigen derived from the finding that lymphocytes from patients with colon carcinoma, which inhibit colony formation of plated colon carcinoma cells, also inhibit fetal gut epithelial cells but not normal adult colon epithelial cells or fetal kidney cells. This finding is concordant with the view that cancer cells sometimes or always express antigens that are normally only to be found in large amounts in fetal tissue. Seen thus, malignancy is a form of dedifferentiation.

In brief, human tumors seem for the most part to be antigenic. Some tumors have demonstrable surface or intracellular antigens that can give a clue to the causative agent. However, for most human tumors, the demonstration of associated antigens is inferred from immunologic tests, and their nature remains unknown.

Tumor Immunogenicity

To entertain validly the concept of a relationship between immunity and malignancy, the demonstration of tumor antigenicity is insufficient. Also needed are data that tumors or tumor-associated antigens are immunogenic in their hosts, that is, that they can indeed elicit immune responses naturally. The immunogenicity of human tumors has been demonstrated in vivo in a number of ways. It has been shown that patients injected with cell-free extracts or membrane extracts of their own tumors often manifest a typical delayed hypersensitivity reaction at the injection site. Histologic examination of biopsy specimens from the skin test sites can show perivascular infiltration of mononuclear cells, a picture consistent with delayed hypersensitivity. A skin window procedure employing fixed cryostat sections of malignant breast tissue can elicit responses consistent with delayed hypersensitivity, but this does not occur with sections of benign breast tissue.

In vitro methods have also been employed to demonstrate that a state of sensitivity to a tumor exists in its host. The macrophage migration inhibition test is a simple assay, and it has shown that the migration of peripheral macrophages was inhibited in response to the soluble antigens of chemically induced sarcomas in guinea pigs that had been immunized with this same extract. Similar reports soon followed for human breast and colon tumors. The significance of these results is that MIF is released from specifically sensitized lymphocytes upon subsequent encounter with sensitizing antigen. Therefore, at least these human tumors can be considered immunogenic. In tumor patients the already mentioned colony inhibition assay substantiates the existence of lymphocytes sensitized to and capable of reacting with tumor cells in a specific manner. Direct cytotoxicity tests, employing cancer patients' lymphocytes with target cells derived from their own tumor or from an allogenic tumor of identical histologic type, have revealed the immunogenicity of colon carcinoma, neuroblastoma, bladder carcinoma, melanoma, breast carcinoma, ovarian and testicular tumors, sarcomas, and head and neck epidermoid carcinomas.

Of course, the question that must now arise is if tumors are indeed antigenic and immunogenic, how can they arise and proliferate. Many explanations have been proposed for the escape of tumors from immune destruction. One postulation is that cells of certain tumors may be only weakly antigenic and may not provide a strong enough stimulus to provoke efficient recognitive and effector processes. For colon carcinoma, the in vitro antitumor immunoreactivity seems inversely correlated with the histologic degree of differentiation of the tumor, thus suggesting that poorly differentiated tumors may provoke less frequent and less intense immune responses and, therefore, can become more widely spread because of this poor degree of immunologic restriction.

A role for tumor antigen as an escape mechanism has been proposed. Papain-solubilized tumor membrane extracts of pooled colon carcinomas can inhibit the cytotoxic effect of sensitized lymphocytes from patients with colon carcinoma. This inhibitory effect is not observed with similar extracts of normal colon cells or melanoma cells, suggesting that an antigen associated with colon carcinoma was involved. In an animal model involving chemically induced hepatomas or sarcomas, tumor cell extracts likewise inhibit lymphocyte cytotoxicity for cultured tumor cells. Interestingly in this situation, soluble extracts of tumors containing embryonic antigens or soluble extracts of embryos themselves also inhibited lymphocyte toxicity, indicating that one of the targets for the sensitized lymphocytes could well be a fetal antigen. Such inhibitory reactions no doubt represent a complex array of events, but it is possible that free tumor antigen could bind to the homologous receptor on sensitized lymphocytes and block their effect in this manner.

A role for antibody has also been postulated in the mechanism by which tumors escape immune attack. Numerous studies originally proposed the phenomenon of the blocking antibody, since sera of tumor patients could inhibit the cytotoxicity in vitro of sensitized lymphocytes for cultured tumor cells. It was believed that this antitumor antibody was bound to the antigenic site of the tumor cell, and thus these sites were masked for recognition by effector lymphocytes. Subsequently, employing a rat hepatoma model, it was shown that antibody alone could not block the attack of sensitized lymphocytes. When soluble tumor antigen was added to the antibody, the blocking phenomenon was in evidence, suggesting that the responsible moiety was an immune complex of antibody and antigen. The specificity of immune complex blocking was shown in that hepatoma extracts blocked only the lymphocyte reactivity to homologous tumors. One study now estimates that nearly 50 per cent of human tumor patients show evidence of circulating immune complexes in their blood, although the identity of the complex constituents is unknown. If immune complexes block human antitumor activity, they could do so by masking the target cell antigen by the antibody portion or by ineffectual combination of the antigen portion with the receptor on the effector lymphocyte.

Another escape mechanism could involve the inappropriate operation of normal suppressive immune mechanisms mediated either by suppressor T-cells or by the inhibitory molecules released by macrophages as they modulate an immune response. It has already been shown that cancer patients with epidermoid cancer of the head and neck regions and with certain other solid tumors possess a population of circulating immune cells, probably macrophages, that paradoxically suppress the immune capacities of their own T-cells. The reason for the appearance of this suppressive function is unclear, but it is possible that it may be a perverted homeostatic response to the display by the tumor cell of self and nonself

antigens. If the antigenic display of the tumor appeared to be predominantly self or if the immune system of the cancer patient did not possess appropriate discriminative capacities, the immune response could be one of tolerance by active suppression rather than immune attack. Defective Ir genes could also be involved. This type of immunologic perturbation has also been observed in animal tumor systems involving virus-induced sarcomas, chemically induced rhabdomyosarcomas, and spontaneous mammary carcinomas.

Interference with the Immune System

If an essential relationship exists between the functions of the immune system and malignancy, one would expect that interference with immunity could result in an increased propensity for the development of malignancy. In fact, this appears true, but with certain reservations.

Neonatal thymectomy renders laboratory rats more susceptible to the development of kidney and bone sarcomas when these animals are inoculated with polyoma virus. Similarly, immunologically impaired animals show an increased number of tumors, with a shorter latent period, after inoculation with simian virus 40 (SV 40). Treatment of mice with antilymphocyte serum (ALS) renders them exquisitely sensitive to the induction of diverse tumors following inoculation with adenovirus or parotid tumors or following inoculation with polyoma virus. Of crucial significance is that the antiviral antibody titers in the ALS-treated animals were similar to those of control animals. Therefore, the increased incidence of tumors could not be due to increased viral replication, since an appropriate B-cell response seemed in evidence. The immunosuppressive effects of ALS on the T-cell system must be implicated. Contrary to these findings is that congenitally athymic mice (nude mice) show no differences in either the latent period or the incidence of local sarcomas or lung adenomas after administration of 3-methylcholanthrene, a potent chemical carcinogen. Therefore, the assumption of a correlation between depressed immune functions and high tumor risk is not absolute. Immunologic control may not be operative in certain malignancies, and this seems to be especially true for those resulting from polycyclic hydrocarbon carcinogens.

The processes associated with aging represent a natural or involutional interference with immunity, because cellular immune vigor wanes with advancing age. The tuberculin skin reactivity of adults decreases significantly after age 55, and the incidence of negative PPD reactions in the older age group cannot be explained by decreased exposure to the tubercle bacillus. Also, nearly 100 per cent of younger individuals should be able to be sensitized to dinitrochlobenzene (DNCB) so that upon a later challenge a typical delayed hypersensitivity reaction should result. Only about 70 per cent of older individuals show such a cellular immune capacity. Co-incidentally perhaps, the risk of developing cancer increases with advancing age, so that by age 90 the chance of developing cancer is 200 times that at age 15. Also, aged mice can show nearly a 100 per cent susceptibility to malignant conversion by the Moloney sarcoma virus, whereas the risk for young adult mice of the same strain can be negligible. Nonimmunologic somatic factors are also operative, however, since age-related changes in target tissue have been demonstrated in laboratory animals. Dimethylbenanthracene-induced carcinomas are three times more frequent in aged skin than in young skin.

The human immunodeficiency diseases were once thought to provide provocative evidence that impaired immunity resulted in an increased incidence of malignancy. Grouping together the registered cases of X-linked agammaglobulinemia, severe combined immunodeficiency, Wiskot-Aldrich syndrome, and ataxia-telangiectasia, it appeared that the incidence of malignancy was an alarming 6 to 10 per cent. However, the preponderance of lymphoid malignancies and the paucity of solid tumors lead to the very real speculation that the immunodeficiency and the lymphoid malignancy may merely reflect fundamental genetic defects that are the cause of both and that no cause and effect relationship exists between the immunodeficiency and the cancer. Indirectly supportive of such a formulation is the analysis of inherited disorders with a high cancer risk but without concomitant immunodeficiency. Fanconi's anemia eventuates in malignancy in about 10 per cent of the cases, with epidermoid carcinomas, hepatomas, and leukemias being observed. Klinefelter's syndrome is associated with an increased risk of leukemia or bronchogenic carcinoma. Xeroderma pigmentosum is associated with frequent skin tumors. To date, no immunologic abnormalities have been described in any of these three diseases. It is possible that malignant transformations occur so frequently in these disorders that even a normal immunologic apparatus is insufficient to restrict tumor growth.

Necessary interference with the human immune system in the form of therapeutic or incidental immunosuppression has resulted in an increased incidence of malignancy. The immunosuppressive measures necessary for successful kidney transplantation are illustrative in this regard. Among 6297 individuals reported to the Renal Transplant Registry of the American College of Surgeons, the risk of developing a lymphoma was calculated to be 35 times greater than normal. Skin and lip epidermoid carcinomas occurred four times more frequently. Other cancers were only 2.5 times more common. These data can be criticized because they represent the combined experience of 30 countries representing a very heterogeneous population. More instructive are the data from the registry of the Scandia Transplant Program in which the 418 Danish patients who received their first and only transplant were followed up for up to 4 years. Twelve tumors occurred in these patients, and a comparison with the expected age, sex, and time-specific incidence rates from the Danish Cancer Registry disclosed a significantly increased tumor incidence. The experience from the University of Colorado Transplant Tumor Registry has disclosed an inordinate number of head and neck tumors. The total world experience seems to justify the assertion that treatment of laryngeal carcinoma by excision and laryngeal transplantation is probably not justified owing to the many complications, one of which is malignancy itself.

There also appears to be an increased incidence of malignancy among individuals receiving chemotherapy. Lymphoreticular neoplasms have been reported in two of 85 patients with rheumatoid arthritis treated with cyclophosphamide. A girl with the nephrotic syndrome developed a malignant mixed müllerian tumor of the cervix after 2 years of cyclophosphamide therapy. Methotrexate therapy for psoriasis has been followed by nasopharyngeal carcinoma in one case and lower extremity metastasizing epidermoid carcinoma in another, both of these tumors representing most unusual complications of psoriasis. Both cyclophosphamide and methotrexate are effective immunosuppressive drugs in humans. Prolonged therapy with cyclophosphamide reduces the number of circulating lymphocytes and depresses the response to the lymphocyte mitogen phytohemagglutinin. Cyclophosphamide and methotrexate can inhibit the cutaneous response to PPD in experimental animals. It is possible that the immunosuppressive effects of such drugs are contributing to the genesis of the malignancy.

Immunoprognosis

Additional support for a relationship between immunity and malignancy derives from the fact that concurrent immune deficits in cancer patients correlate directly with poor prognosis. Numerous studies of patients with colon, breast, and head and neck carcinoma have shown that negative DNCB skin tests correlate with inoperability or early recurrence. DNCB positivity in patients with epidermoid head and neck tumors correlates with good responses to radiotherapy. A morphologic measurement of immunologic activity in lymph nodes regional to such tumors has also been shown to be of prognostic value. Patients whose lymph nodes show evidence of immunologic stimulation seem to have 5-year survival rates nearly twice as great as those who do not. It thus appears that measurement of a patient's cellular immune capacities may help to identify those at risk for not responding optimally to conventional modes of therapy. In this manner, therapy may be better individualized, and developing immunotherapeutic modalities may be employed when indicated.

Large Molecule Therapy

The definition of immune processes in molecular terms is just beginning. With this definition have come exciting insights that the large molecules of immunocompetent cells may be administered to compensate for an immunologic aberration and thus result in the manipulation of disease. Large molecule therapy will most certainly complement small molecule pharmacologic therapy.

Thymosin

In addition to its vital role in ontogeny for the differentiation of T-cells, the thymus serves an important endocrine function. The first evidence that the thymus secretes humoral substances came from experiments with thymectomized animals that received a transplanted thymus gland in a cell-impermeable chamber. These animals did not experience the lymphocyte depletion characteristic of the thymectomized state. Substantiating evidence came from further experiments demonstrating the reconstitution of immunologic responsiveness in neonatally thymectomized animals using thymic epithelium in a cell-impermeable chamber. The epithelial cells of the thymus are capable of secreting one or more humoral agents that appear to play a role in the expression of immunity.

One of these agents, thymosin, can be partially purified from bovine material and from humans. In vitro, thymosin can convert murine stem cells of bone marrow origin into mature T-cells expressing the teta antigen and capable of reacting in various assays of cellular immunity characteristic of mature, differentiated T-cells. Thymosin activity is present in human serum and begins to decrease at puberty, the time at which the thymus begins involution. In patients with myasthenia gravis who undergo therapeutic thymectomy, thymosin activity disappears from the serum within 24 hours. There is a suspicion that the decreased cellular immunity seen with advancing age may be due in part to the decrease of the endocrine functions of the thymus, although the visible effects may not be immediately dramatic because of the long-lived peripheralized memory cells.

Thymosin is now being employed clinically in selected immunodeficiency diseases. Children with severe combined immunodeficiency have been treated with thymosin with

uniformly negligible results. Since children appear to lack the progenitor cell on which thymosin can exert its effects, this is not surprising. Other immunodeficiency diseases involving thymic dysfunction such as the Nezelof syndrome and ataxia-telangiectasia appear to improve with thymosin administration. These disorders do not involve the severe stem cell problem seen in severe combined immunodeficiency, and, therefore, this type of therapy is intellectually appealing.

Cancer patients are just beginning to be treated with thymosin. A rationale for this mode of therapy is that the peripheral lymphocytes of cancer patients, when incubated with thymosin in vitro, show an increase in the number of cells that are capable of spontaneously rosetting with sheep red blood cells. The supposition here is that somehow the T-cells of the cancer patient are not capable of mature function since some of them apparently are not expressing a marker characteristic of mature T-cells. Although these clinical trials have only recently begun, the claim has already been made that thymosin therapy has allowed the conversion of negative delayed skin tests to positive. The relationship of this in vivo demonstration of increased cellular immunity to tumor survival is awaited. It is also interesting that thymosin administered in cases of chronic lymphocytic leukemia (often a malignant B-cell expansion) can result in decreased peripheral lymphocyte counts. Some wish to explain this phenomenon by postulating a role for thymosin in maintaining the function of suppressor T-cells.

Interferon

As mentioned previously, interferon can be produced by a variety of cells in addition to lymphocytes. Interferon preparations have been derived from a number of animal species, and despite many residual impurities, this substance demonstrates powerful biologic activities, indicating that it has one of the highest known specific activities of biologic materials.

The originally described property of interferon was its ability to act intracellularly to limit the replication of viruses. However, certain other intracellular parasites such as rickettsiae and protozoa are subject to its effects. Interferon inhibits the synthesis of viral proteins so that mature viral particles cannot be assembled. Interferon's clinical antiviral properties have been demonstrated in numerous situations. It can decrease the number of successful primary smallpox vaccinations, and it shows a prophylactic efficacy in influenza infections. Patients with cancer or those who have received organ transplants experience a decreased incidence of viral infections while undergoing interferon therapy. Interferon can produce beneficial effects in patients with herpes zoster infections, and patients who have undergone bone marrow transplantation and have developed cytomegalovirus infections can show a significant decrease in cytomegalovirus titers after administration of interferon.

Interferon has antitumor activity also. It was originally demonstrated that oncogenic viruses were also susceptible to interferon. Virus-induced leukemias and sarcomas showed a delayed appearance or a retarded progression in experimental animals after interferon therapy. The spontaneous leukemia of AKR mice could undergo resolution after treatment with interferon. Curiously, interferon is also active against tumors that have no known viral cause. Interferon can protect laboratory animals against the inoculation of lethal doses of tumor cells or against the induction of tumors by chemical carcinogens. Interferon appears to exert its antitumor action in two main ways. It has direct effects on the tumor cell, being able to

inhibit tumor cell division or to change the surface properties of tumor cell membranes. Interferon also works through the immune system. It can modulate T- and B-cell function, enhance macrophage phagocytic activity, and alter serum complement levels. Already, interferon is being used in the adjunctive treatment of human cancer, and an initial trial in patients with osteogenic sarcoma is most promising.

Transfer Factor

The mysterious transfer factor of Lawrence is a dialyzable material of viable leukocytes. If obtained from immune cells of donors sensitized to a certain antigen and injected into an unsensitized individual, a state of delayed hypersensitivity, as determined by skin test reactivity, can be induced to that same antigen. It appears that only cellular immune responses can be transferred in this manner. That transfer factor confers specific cellular immunity is difficult to reconcile with the small size of the molecule. Furthermore, some recipients of transfer factor have developed sensitivity to antigens to which the donor of the transfer factor was not sensitive. Transfer factor may indeed act quite independently by enhancing the induction of expression of cellular immune capacities.

Some patients with chronic mucocutaneous candidiasis benefit from transfer factor. Patients with coccidiomycosis can likewise show clinical improvement with concomitant conversion of skin test reactivity to coccidioidin in vivo and lymphocyte mediator production to coccidioidin in vitro. Transfer factor has also been used in the immunotherapy of viral infections and seems to be of significant benefit in measles pneumonia.

Since the question of specificity is not yet resolved, it is difficult to decide whether transfer factor must be prepared from donors who are sensitized or immune to the antigen under treatment or whether pooled normal human transfer factor prepared from random donors can be used, based on the assumption that it is some type of nonspecific immunomodulator. This question is brought into bold relief when considering transfer factor therapy for immunopathic diseases of uncertain cause. Pooled normal human transfer factor has resulted in varying but generally disappointing results in the treatment of multiple sclerosis, a disease with circumstantial evidence to implicate the measles virus as its cause. Perhaps, transfer factor prepared from donors exquisitely sensitive to the measles virus may have been more efficacious. The attempted treatment of malignant disease is even more problematic in this regard. Since household contacts of some tumor patients can demonstrate in vitro cellular immunity to that particular tumor, these individuals could be logical donors of transfer factor if one wished not to ignore the possible specificity of its effects. In fact, treatment of patients with osteogenic sarcoma with transfer factor prepared from such household contacts can result in an increase of in vitro cytotoxicity against the tumor cells and in a lymphocyte infiltration around the tumor. Patients receiving nonspecific transfer factor, however, show evidence of declining cytotoxicity, which can be reversed when specific transfer factor is used.