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Immunodeficiency Diseases and Tumor Immunobiology

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IMMUNODEFICIENCY DISEASES AND TUMOR IMMUNOBIOLOGY

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INTRODUCTION

Basic progress toward an improved understanding of the relation of structure to function of cells, tissues, and organs of the lymphoid system has taught us about the significance of the immune apparatus in the pathogenesis of many disease processes. The classic definition of immunity as being an exemption from disease has traditionally been related to resistance to infection. Until the turn of the century, the immune system was considered to have evolved as a homeostatic mechanism specifically directed against infectious agents. It is now recognized that the immune system participates in many processes of tissue injury elicited by hypersensitivity reactions against a great variety of innocuous substances. These immunologically mediated hypersensitivity reactions of tissue injury are divided into four major categories: (1) The immediate-type of hypersensitivity reactions mediated by the humoral immune system. (2) The delayed-type of hypersensitivity reactions which are manifestations of cellular immunity. (3) The immune complex diseases which result from the deposition of circulating soluble antigen-antibody complexes in the walls of blood vessels and the glomeruli. (4) Autoallergic disorders which may occur as a consequence of immunologic reactivity against the host's own tissue components. These hypersensitivity reactions will be discussed in greater length in Chapters 17 and 18.

This chapter deals with the immunopathologic

mechanisms which underlie certain abnormalities of the lymphoid system. We will discuss those primary immunodeficiency disorders due to a developmental defect of the lymphoid system as well as the immunodeficiency states secondary to various diseases.

In the section on tumor immunology, malfunction of immunologic processes in patients with malignant disease will be analyzed in terms of the surveillance function of the lymphoid system of malignant growth.

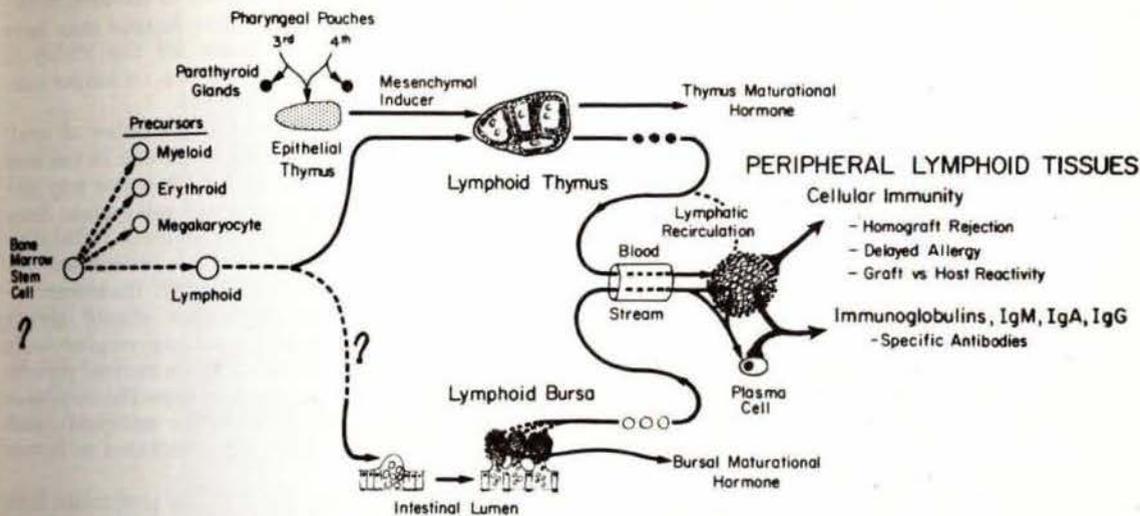
IMMUNODEFICIENCY DISEASES

Development of the Lymphoid System

The hematopoietic system develops from hematopoietic stem cells residing at different locations during ontogenesis; these locations are in the primitive blood islets of the yolk sac, the fetal liver, and finally in the bone marrow. These stem cells can proliferate and differentiate along the hematopoietic pathways of the erythroid, monocytic, granulocytic, megakaryocytic, or lymphoid systems.

In the lymphoid system, the lymphoid stem cells may differentiate to form two lymphocyte populations: the T lymphocytes, which are dependent upon the thymus, and the B lymphocytes, which are dependent upon an undefined bursa equivalent (Fig. 5-1). Under the influence of the inductive microenvironments of the

THYMUS SYSTEM DEVELOPMENT



BURSAL SYSTEM DEVELOPMENT

Figure 5-1 Two-component concept of the lymphoid system. Lymphoid stem cells may proliferate and differentiate under influences of the thymus or a bursa equivalent to give rise to populations of T and B lymphocytes, respectively. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)

thymus cortex and medulla, the prethymic lymphoid stem cells proliferate and differentiate into T lymphocytes (T cells). The lymphocytes which reside in the thymus and which have different stages of lymphoid development are called thymocytes. It is not certain whether differentiation of a thymocyte into a T cell takes place within the thymus before emigration or in the peripheral tissues. Recent experiments with mice indicate that certain medullary thymocytes have properties similar to peripheral T lymphocytes. Further, it appears that subpopulations of T lymphocytes exist, each with a different stage of immunologic development, life span, and immunologic function.

Upon immunogenic stimulation, certain T cells can proliferate and differentiate to become sensitized T cells. These sensitized T cells can be activated by antigen to become "killer" lymphocytes, which produce soluble factors called lymphokines and give rise to the so-called amplification system of cellular immunity. T cells have also been shown to interact synergistically with B cells in the immune response of mice and a limited number of other species, but not in man. These T lymphocytes may express immunologic memory.

T lymphocytes are found in the deep cortical regions of lymph nodes, the periarteriolar sheaths of splenic follicles, the diffused lymphoid

tissue of the gastrointestinal tract, the thoracic duct lymph, and the circulating blood.

In the chicken, the organ responsible for the differentiation of lymphoid stem cells into B lymphocytes (B cells) is the bursa of Fabricius. It has yet to be identified in the mammalian species. The appendix, Peyer's patches, and gut-associated lymphoid tissue as well as tonsils, bone marrow, and even skin have been implicated as the mammalian bursa equivalent; however, these postulates lack sufficient experimental evidence.

There are two categories of B cells: the non-secreting and the secreting B cells. B cells of the first group are capable of synthesizing antibody but are not specialized for antibody secretion in large amounts. These cells carry immunoglobulin receptors for both light and heavy chains and, in mice, also have receptors for C3. They can be found in the far cortical areas of lymph nodes, the perifollicular zones of the malpighian corpuscles of the spleen, and the circulating blood.

Upon interaction with antigen, nonsecreting B cells can transform into secreting B lymphocytes or plasma cells. These cells are specialized for production and secretion of antibody. They can be found in the medullary cords of lymph nodes, in the red pulp of the spleen, in the lamina propria of the gastrointestinal tract and secretory glands, in the interstitial tissue of the bone marrow, and, occasionally, in the peripheral blood and lymph.

The two-component concept of the lymphoid system implies that there are two basic syndromes of immunodeficiency: one, a deficiency of humoral immunity as a consequence of an abnormality of the B cell system, and the other, a defect of the T cell system of cellular immunity. Patients with immunodeficiency diseases manifest parts of each syndrome, depending upon the type and extent of their deficiency. Careful diagnostic evaluation of both B and T cell systems is mandatory in the proper management and treatment of the disease in these patients.

Evaluation of the T Cell System

Generally, a T cell deficiency is indicated with increased susceptibility to infection by fungi, viruses, atypical acid-fast organisms, and some of the so-called lower-grade pathogens. Causative organisms include *Candida albicans*, vaccinia and mumps viruses, Mycoplasma, Staphylococcus, and *Pneumocystis carinii*. On the other hand, fewer problems are encountered with high-grade encapsulated pathogens, such as Pneumococcus, Haemophilus, Streptococcus, and Meningococcus.

Pneumocystis carinii deserves special attention, since it frequently causes severe pulmonary disease in patients with immunodeficiency. In these patients, pneumocystis pneumonia shows characteristic accumulations of foamy, pink-staining exudates containing many pneumocystis organisms (Fig. 5-2A and B). This protozoan produces disease in patients with B and T cell deficiencies and has also caused plasma cell pneumonia epidemics in neonates and premature and debilitated infants in central Europe. The diagnosis of pneumocystis infections in patients with immunodeficiency disease is hampered by the absence of any demonstrable specific immune response. Demonstration of specific antibodies in close relatives of the patient may provide an indication of pneumocystis infection; otherwise, a lung biopsy is a better diagnostic measurement. Early pneumocystis infection can be effectively treated with pentamidine isethionate.

The laboratory evaluation of the T cell system in patients with suspected immunodeficiency is given in Table 5-1. An effective test for the analysis of the T cell population is a quantitation of the responsiveness of peripheral blood lymphocytes or lymph node cells to blastogenic stimulation with phytohemagglutinin (PHA). The capability to develop delayed allergic reactions may be analyzed by the ability to develop contact allergy after stimulation with dinitrochlorobenzene (DNCB). Skin tests for delayed hypersensitivity to ubiquitous antigens such as mumps, *Candida*, Trichophyton, tuberculin, histoplasmin, and streptokinase-streptodornase may be of little value in young children who have had little op-

portunity to develop cell-mediated immune responses to these antigens. However, negative skin tests to *Candida* antigens in children suffering from infection with this fungus may have diagnostic usefulness. Tests for the ability to reject allogeneic skin grafts are no longer commonly used.

Morphologic analysis of the number of small lymphocytes in peripheral blood and in the deep cortical areas of biopsied lymph nodes may provide some quantitation of the T cell system. Since T lymphocytes do not have immunoglobulin receptors on their cell surface, membrane immunofluorescence methods with fluorescent labeled antiglobulin antibodies should give a higher ratio of positively staining lymphocytes in T cell deficient patients than in normal persons. Although T cells in mice have specific membrane markers (e.g., theta and TL antigens), such markers have not been demonstrated on human T cells.

Several tests for lymphokine production have been developed which are of potential usefulness in the evaluation of the amplification system of cellular immunity. In particular, the capillary test for migration inhibitory factor has become a routine procedure in many laboratories.

Evaluation of the B Cell System

A deficiency of the B cell system is generally indicated with frequent occurrences of infection causing pneumonia, otitis media, conjunctivitis, meningitis, and sepsis. Causative microorganisms include Pneumococcus, Streptococcus, Haemophilus, Meningococcus, *Pseudomonas aeruginosa*, hepatitis virus, and *Pneumocystis carinii*. These patients experience fewer problems with handling of Staphylococcus, most enterobacteria, mycobacteria, fungi, and viruses.

Most laboratory tests for evaluation of the B cell system are carried out on the products of B lymphocytes, i.e., the antibodies and immunoglobulins (Table 5-2). Electrophoretic and immunoelectrophoretic analyses of serum and secretions provide qualitative information about the presence or absence of the various immunoglobulins, as well as the presence of homogeneous immunoglobulin populations seen in monoclonal gammopathies.

A variety of immunologic methods are currently available for determining immunoglobulin levels. Most commonly used is the radial immunodiffusion technique by which the test serum is allowed to diffuse in a layer of agar gel containing antiserum specific to one of the immunoglobulins. The concentration of each immunoglobulin is determined by comparing the diameter of the precipitin ring with that given by an immunoglobulin standard solution of defined concentration. When special precautions are

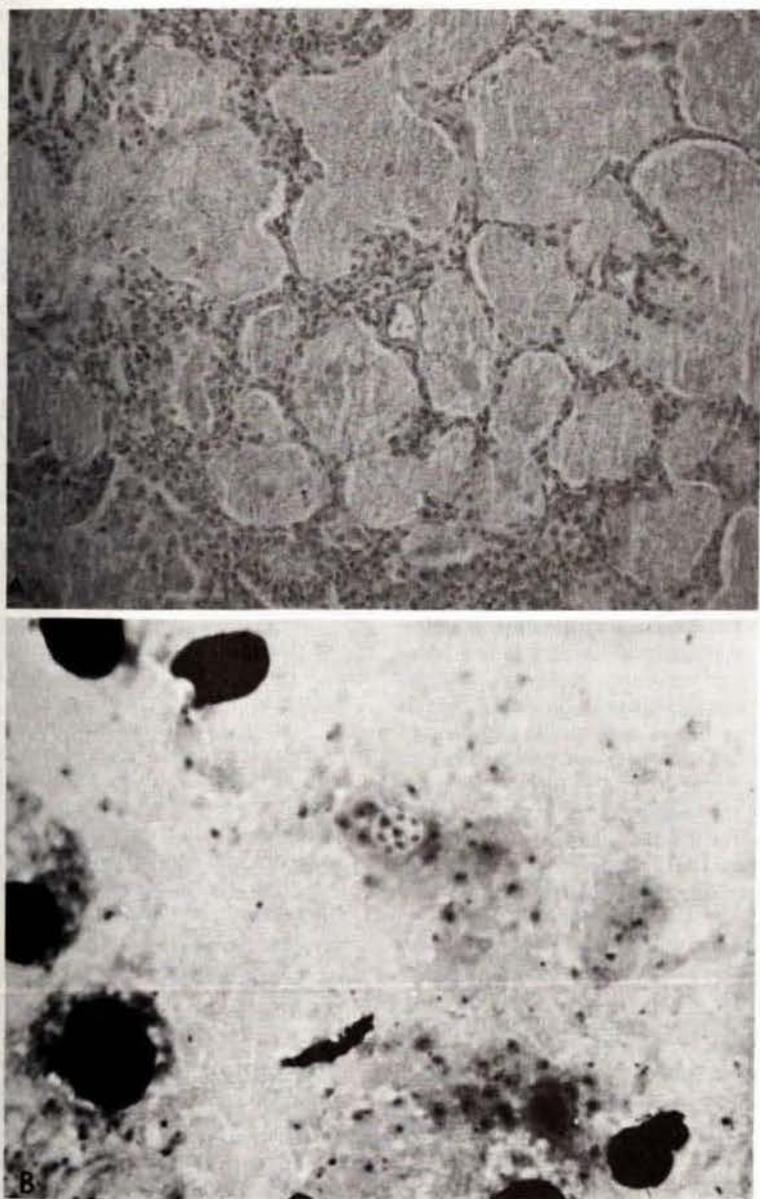


Figure 5-2 Pneumocystis pneumonia in an agammaglobulinemic patient. A, Eosinophilic accumulations in pulmonary lesion. B, *Pneumocystis carinii* organisms in pulmonary exudate. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)

TABLE 5-1 LABORATORY EVALUATION OF T CELL FUNCTION

1. Microbiological studies, history of infections.
2. Quantitation of PHA-induced lymphocyte blastogenesis.
3. Skin tests for delayed hypersensitivity to ubiquitous antigens.
4. Development of contact allergy to DNCB.
5. Blood lymphocyte count.
6. Allogeneic skin graft rejection.
7. Histologic analysis of lymphocyte populations in thymus-dependent areas of lymph nodes.
8. Immunoglobulin receptors on lymphocytes.
9. Amplification of cellular immunity, MIF test.

taken to ensure that the immunoglobulins in the standards and the sera are not split or aggregated, the radial immunodiffusion method can be used to measure, with some accuracy, the levels of IgG, IgM, IgA, and IgD. Serum IgE levels are measured with the radioimmunoassay method. The normal values for each of these immunoglobulins are given on p. 101. Caution should be given in the evaluation of immunoglobulin levels in adult serum, since they may vary greatly from individual to individual and are related to age, sex, environment, and racial background. No threshold for values can be set for the diagnosis of immunoglobulin deficiencies unless associated with clinical symptoms. Reliable measurements cannot be made with such proteins as low molecular weight IgM and secretory IgA unless a standard immunoglobulin is available in the same form.

Special attention should be given to analysis of the subclasses of immunoglobulins. Using appropriate antisera, the levels of immunoglobulin subclasses are measured by radial immunodiffusion or direct hemagglutination-inhibition tests. The approximate percentages in American Caucasians of normal serum IgG are IgG₁, 66 per cent; IgG₂, 23 per cent; IgG₃, 7 per cent; and IgG₄, 4 per cent. The importance of determining immunoglobulin subclasses has been demonstrated in certain patients with normal total IgG serum levels, but lacking one of the IgG subclasses, and showing great susceptibility to pneumococcal and other high-grade, extracellular, pyogenic pathogens, similar to patients with generalized B cell immunodeficiency diseases.

Humoral immune function of the B cell system can be evaluated by tests for existing antibody to antigen to which man is commonly exposed or by humoral antibody assays following active immunization. Antibodies to ubiquitous antigens include type A and B isohemagglutinins and antibodies to infectious agents, e.g., antistreptolysin O, antiviral antibodies and agglutinins of typhoid, paratyphoid, brucella, diphtheria,

tetanus, *Candida*, and *Staphylococcus*. The antigens used for active immunization should be proteins as well as polysaccharides. Quantitation of antibody responses may be carried out following injection of poliovirus vaccines, diphtheria, tetanus, and pertussis toxoid, and pneumococcal or meningococcal polysaccharide antigens. The use of live vaccines (e.g., *Bacillus of Calmette-Guerin* (BCG) or attenuated viruses) should always be avoided. However, more than just the total amount of antibody produced should be considered. The induction, logarithmic, plateau, and decline phases of antibody production should be measured whenever possible. The normal maturation of the immune response (from predominantly IgM to predominantly IgG) ought to be followed.

Absence of low serum levels of one or more immunoglobulins may have three possible causes: (1) absence or decreased numbers of B cells in the circulation and lymphoid tissue, (2) a defect in immunoglobulin secretion by B cells, and (3) an increased rate of immunoglobulin catabolism. The following tests need to be carried out to distinguish between these possibilities. Determination of lymphocyte membrane immunofluorescence with fluorescent antibodies against the various immunoglobulins can be used to analyze the B cell population in the circulation as well as in the lymph nodes. In addition, one can look for the presence of germinal centers and plasma cells in lymph nodes. The evaluation of immunoglobulin catabolism can be determined by measurement of the rate of disappearance of radioactivity from the circulation following injection of radio-labeled immunoglobulins.

A complete evaluation of the B cell system should include analysis of the amplification systems belonging to the various types of antibodies. For instance, analysis of complement-fixing antibodies can be carried out to detect those types of

TABLE 5-2 LABORATORY EVALUATION OF B CELL FUNCTION

1. Microbiological studies, history of infections.
2. Electrophoresis and immunoelectrophoresis of serum.
3. Concentration of immunoglobulin classes and subclasses in serum and secretions by radial immunodiffusion.
4. Tests for antibodies to ubiquitous antigens.
5. Quantitation of humoral immune response.
6. Immunoglobulin receptors on lymphocytes by membrane immunofluorescence.
7. Histology of lymph node biopsies, germinal center formation.
8. Analysis of immunoglobulin catabolism.
9. Amplification of humoral immunity. Complement system.

IgG antibody which are unable to activate the complement system. Evaluation of the complement system may also be necessary to determine the levels of complement components and some of the homeostatic control mechanisms of the complement system, such as C1 esterase inhibitor.

Primary Immunodeficiencies

Primary immunodeficiency results from a failure to produce the effectors of the immune response, i.e., antibodies and sensitized lymphocytes. Excluded from this definition are the hypercatabolic states and disorders in the amplification systems, such as the complement deficiencies. Secondary immunodeficiencies may occur in patients with a variety of diseases.

The variability of immunologic findings presents a major difficulty in classification of the primary immunodeficiencies. Often neither etiologic nor functional or structural considerations, taken alone or together are satisfactory for adequate classification of immunodeficiency diseases in man. Well-characterized diseases associated with primary immunodeficiency are listed in Table 5-3. However, the majority of patients with immunodeficiency cannot be unequivocally classified and are therefore grouped under the heading of variable immunodeficiency. The probable levels at which various primary immunodeficiency diseases interfere with normal lymphoid development are shown in Figure 5-3. Only a few will be selected for discussion.

Patients with infantile, sex-linked agammaglobulinemia (Bruton's agammaglobulinemia)

have an immunodeficiency exclusively involving the B cell system while the functions attributable to the T cell system appear to be intact. Concentration of each of the immunoglobulins in serum or secretions is very low or absent, and humoral antibody responses are absent even to repeated injections of potent antigens. Histologic studies show that lymph nodes and spleen of these patients lack germinal centers, and that plasma cells are absent from lymph nodes, spleen, bone marrow, and connective tissue including the lamina propria (Figs. 5-4A and B, 5-5A and B). The tonsils have poorly developed lymphoid elements which lack follicular components (Fig. 5-6A and B). By contrast, these patients have normal or nearly normal levels of circulating lymphocytes, normal numbers of lymphocytes in the thymus-dependent regions of lymph nodes and spleen, and a normal thymus. Membrane immunofluorescence methods show absence of immunoglobulin receptors on these lymphocytes.

These patients usually have recurrent bacterial infections which begin during the second six months of life. They show extraordinary susceptibility to infection with certain extracellular pyogenic pathogens like Pneumococcus, Streptococcus, and Haemophilus, but infections with other organisms like Staphylococcus and Pseudomonas occur only occasionally. Many patients develop pneumocystis infections. They suffer repeatedly from acute episodes of otitis, sinusitis, skin infection, conjunctivitis, and pulmonary disease. Such patients are also prone to septicemia and meningitis. In striking contrast, these patients can resist infections by many other microorganisms including viruses, fungi, and most enterobacteria, even when they appear to produce little or no antibody to these microorganisms. They seem unable to resist infection with hepatitis virus, and such infections usually cause either massive liver destruction or chronic active hepatitis with steady progression to complete destruction of the liver. Patients with sex-linked agammaglobulinemia are prone to develop connective tissue diseases such as arthritis, dermatomyositis, and diffuse vasculitis. Furthermore, they often develop malignant conditions, especially in the lymphoreticular and gastrointestinal tissues.

Certain selective immunoglobulin deficiencies have been described which seem to be associated with defective development of a single immunoglobulin class. One of the most common primary immunodeficiency diseases is the isolated (or selective) deficiency of IgA which affects 1 out of 700 persons. Most patients are clinically asymptomatic but some may have recurrent gastrointestinal infections and a spruelike syndrome. The incidence of rheumatoid arthritis, lupus erythematosus, tenosynovitis, and other

TABLE 5-3 SELECTED PRIMARY IMMUNODEFICIENCY DISEASES*

Disorder	Suggested Cellular Defect		
	B Cells	T Cells	Stem Cells
Infantile sex-linked agammaglobulinemia (Bruton's)	+		
Selective immunoglobulin deficiency (IgA)	+		
Thymic hypoplasia (DiGeorge syndrome)		+	
Combined immunodeficiency	+	+	+
Ataxia-telangiectasia	+	+	
Wiskott-Aldrich syndrome	+	+	
Immunodeficiency with thymoma	+	+	
Variable immunodeficiency (unclassified)	+	(+)	

*A complete classification is given in Fudenberg, H., et al.: Pediatrics, 47:927, 1971.

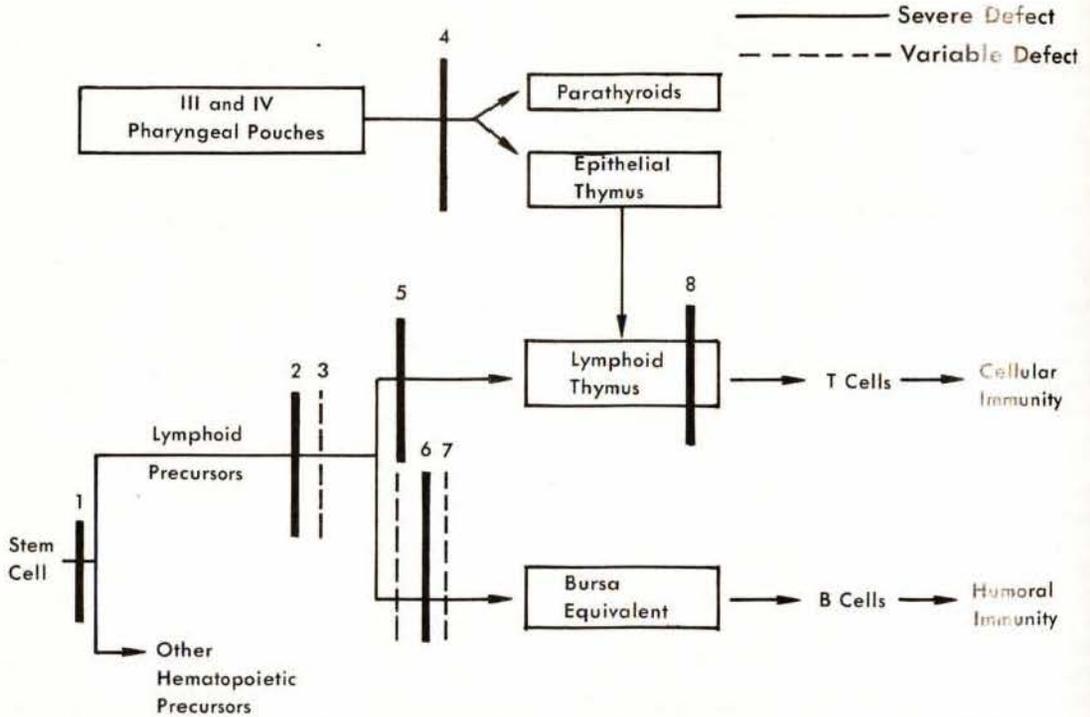


Figure 5-3 Possible sites of defect in the development of the lymphoid system of patients with various types of primary immunodeficiency. (1) Immunodeficiency with generalized hematopoietic hypoplasia, reticular dysgenesis. (2) Autosomal recessive combined immunodeficiency. (3) Sex-linked and sporadic types of immunodeficiency. (4) Thymic hypoplasia (DiGeorge). (5) Ataxia-telangiectasia. (6) Infantile sex-linked agammaglobulinemia. (7) Selective immunoglobulin deficiency (IgA), transient hypogammaglobulinemia of infancy. (8) Thymus dysplasia with normal immunoglobulins (Nezelof).

mesenchymal diseases is higher in patients with selective IgA deficiency. A number of patients with low levels of IgA produce anti-IgA antibodies which lead to decreased survival rate of serum IgA. Blood products containing IgA given to these individuals may result in anaphylactic reactions. Selective IgA deficiency causes low levels of IgA in serum as well as in secretions. The two subclasses, A_1 and A_2 , are usually reduced in equal proportions. However, in certain patients, low serum IgA levels are accompanied by normal concentrations of secretory IgA. The reverse situation may be present in other patients with IgA deficiency.

Selective IgE immunoglobulin deficiencies have been described in children and are associated with frequent episodes of respiratory infections developing into a progressive sinopulmonary disease.

Severe immunodeficiency disease of the thymus-dependent system is present in patients with thymic hypoplasia (DiGeorge syndrome). In these patients there is a congenital arrest in the development of the thymus and parathyroids from the epithelial anlage derived from the III and IV pharyngeal pouches. Such infants with

hypocalcemic tetany at birth are unable to survive because they lack the capacity to develop the T cell system and to express cell-mediated immunity. Circulating lymphocyte counts are usually near normal at birth but soon decline to a significantly low level. These patients fail to express any of the functions of cellular immunity and have a high susceptibility to fungal and viral infections. By contrast, their levels of immunoglobulin are normal, plasma cells are present in normal numbers in the lymphoid tissues in bone marrow, and the thymus-independent regions of lymph nodes and spleen are well preserved. The thymus-dependent, deep cortical areas of lymph nodes and periarteriolar sheaths of splenic follicles are depleted of lymphocytes (Fig. 5-4C). Infections usually begin early in life and may be caused by fungi, viruses, atypical acid-fast organisms, low-grade and high-grade encapsulated bacteria, and *Pneumocystis carinii*.

Severe combined immunodeficiency disease affects both B and T cell systems. The autosomal recessive variant of this lymphocytic agammaglobulinemia has been called Swiss type agammaglobulinemia. These patients have severe cellular defects in the development of hu-

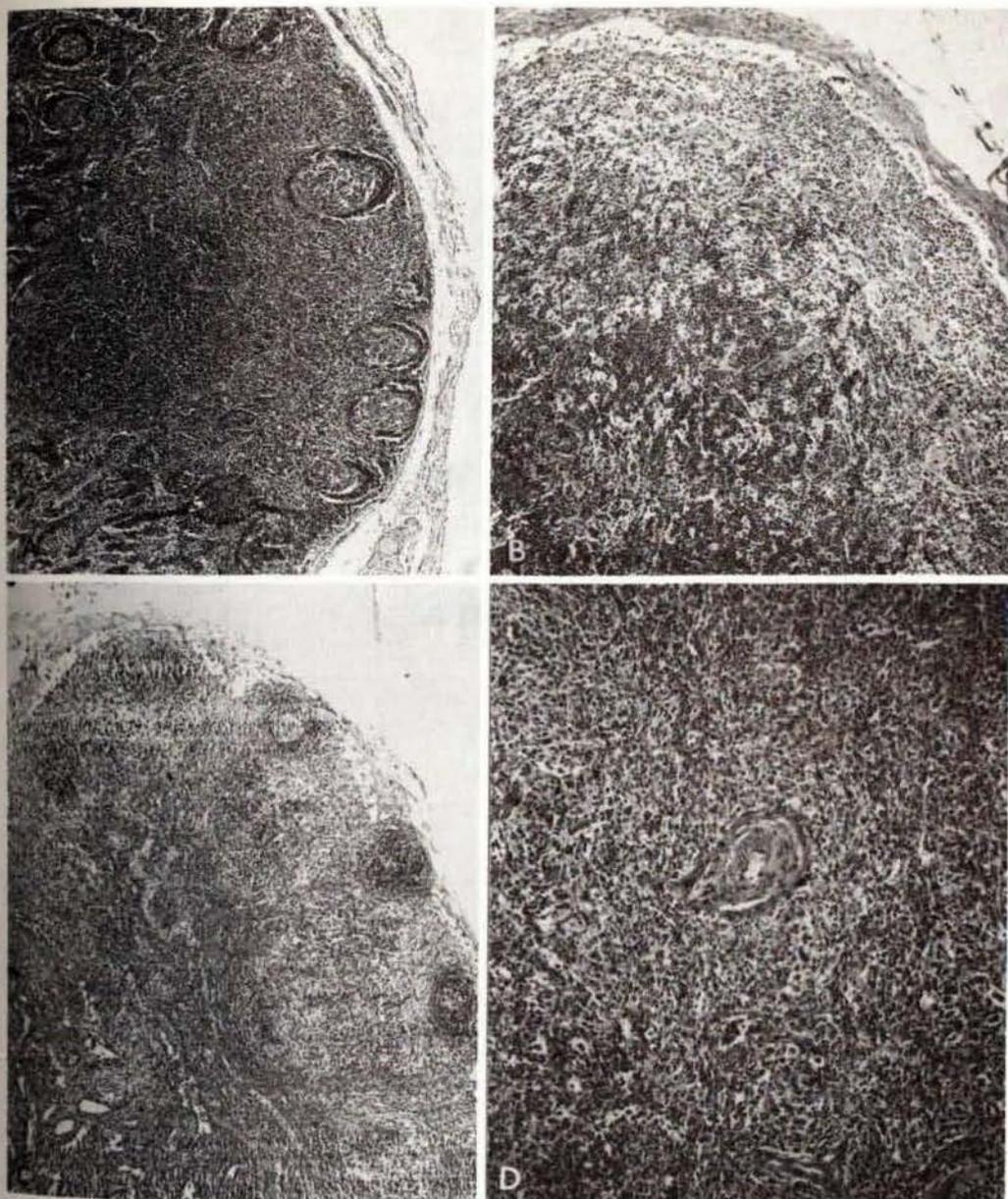


Figure 5-4 Lymph node histology in primary immunodeficiency. *A*, Stimulated lymph node of a normal 11-month-old child. Well-developed germinal centers and abundance of plasma cells. The cortical and deep cortical regions are densely populated by lymphocytes. *B*, Lymph node from a boy with infantile sex-linked recessive agammaglobulinemia 5 days after secondary antigenic stimulation. Absence of cortical germinal centers and plasma cells, dense populations of small lymphocytes in the deep cortical regions, and lymphocyte depletion in cortex. *C*, Lymph node from a patient with thymic hypoplasia. The thymus-dependent deep cortical regions are depleted of small lymphocytes. Lymphoid follicles, germinal centers, and plasma cells are present. *D*, Lymph node from a child with autosomal recessive combined immunodeficiency disease. Severe depletion of lymphocytes and plasma cells accompanied by histiocytosis and stromal hyperplasia. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)

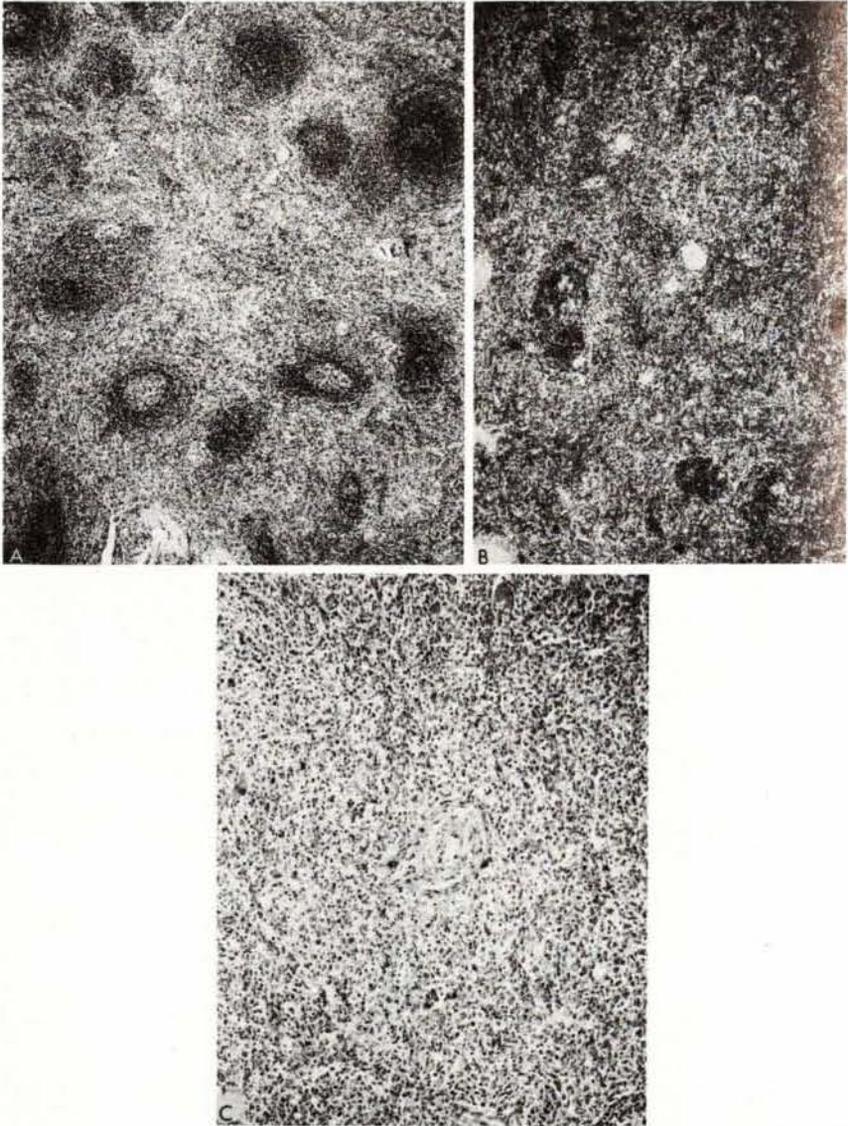


Figure 5-5 Histology of spleen in primary immunodeficiency. *A*, Spleen of a child who died of congenital heart disease at 3 months of age. Note the normal dense lymphoid cellularity of the malpighian follicles and several germinal centers. *B*, Spleen from child with sex-linked agammaglobulinemia. Note the primitive development of lymphoid follicles and absence of germinal centers and plasma cells. *C*, Severe depletion of lymphocytes and plasma cells in spleen from patient with autosomal recessive combined immunodeficiency disease. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)

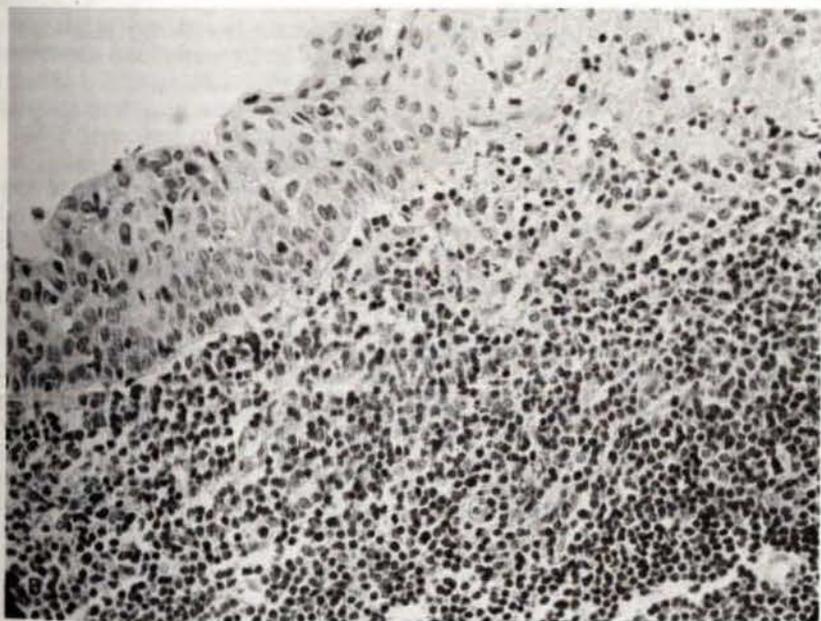
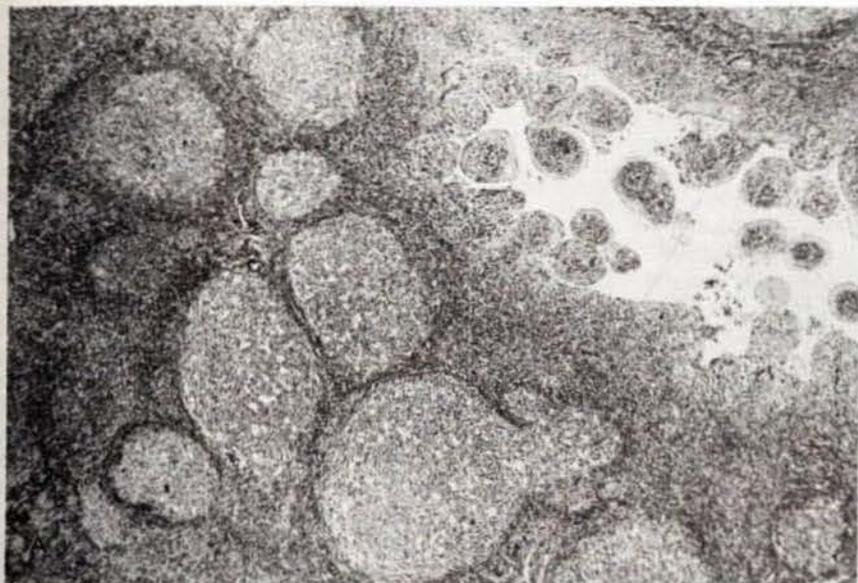


Figure 5-6 A, Normal tonsillar tissue with abundance of lymphoid elements. B, Tonsil from a patient with infantile sex-linked agammaglobulinemia. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)

moral as well as cell-mediated immunity as a consequence of a lymphoid stem cell defect. Very young infants are affected by this disease and suffer recurrent infections with a great variety of microorganisms. They generally succumb during the first year of life to low-grade, opportunistic pathogens such as *Pseudomonas*, *Staphylococcus*, the common enterobacteria, *Pneumocystis carinii*, and *Candida albicans*. If exposed to measles virus, they may die of a characteristic giant cell pneumonia (Hecht's pneumonia); they do not show the characteristic measles rash. Also, progressive, generalized vaccinia will occur after dermal smallpox vaccination.

Serum levels of all immunoglobulin classes are either absent or extremely low. The thymus is hypoplastic and lacks lymphoid cells and Hassall's corpuscles (Fig. 5-7). These patients have severe lymphopenia and an absence or marked deficiency of lymphocytes in the thymus-dependent as well as the thymus-independent regions of peripheral lymphoid tissues (Fig. 5-4C and D).

In addition to the Swiss type of agammaglobulinemia, a large number of patients have lymphopenic immunodeficiency syndrome which varies greatly in the clinical and laboratory manifestations. Some of these patients are clearly of sex-linked recessive inheritance, others seem to be inherited as autosomal recessive traits, and still others have thus far not revealed any hereditary patterns. Laboratory evaluation of both T and B cell systems show variability of immunodeficiency. Some patients with these forms of combined immunodeficiency possess nearly normal amounts of IgM, little IgG, and no IgA.

Different degrees of defective development of peripheral lymphoid organs and thymus have also been observed.

Immunodeficiency of the B cell system, and especially of the T cell system, is encountered in ataxia-telangiectasia (Louis-Bar syndrome). These patients develop a progressive cerebellar ataxia which usually begins during the first few years of life. They often have recurrent pulmonary infections and develop prominent telangiectases in the sclerae, the skin of the eyelids, the ear lobes, the neck, and the popliteal and antecubital spaces. These patients have variable degrees of deficiency of the cell-mediated immune responses. In some instances they show deficient antibody production to certain relatively weak immunogens. A large percentage have low levels of IgA and IgE as a consequence of decreased rate of synthesis of the immunoglobulins. Several patients have an increased rate of IgA catabolism because of the presence of anti-IgA antibodies. Histologically, the thymus-dependent regions of the peripheral lymphoid tissue show a depletion of small lymphocytes. The thymus is small, lacks Hassall's corpuscles and is poorly organized into a cortex and medulla (Fig. 5-8). Female children with ataxia-telangiectasia frequently show ovarian dysgenesis. The etiology of this autosomal recessive disease is not well understood, but may be due to a neuroendocrine abnormality. These patients have a relatively high susceptibility to malignant disease, in particular, lymphosarcoma, reticulum cell sarcoma, or reticuloendotheliosis.

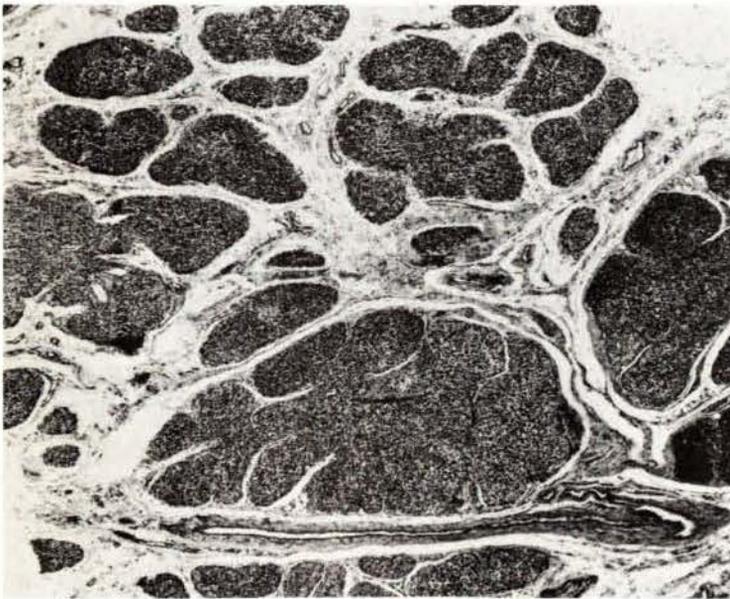
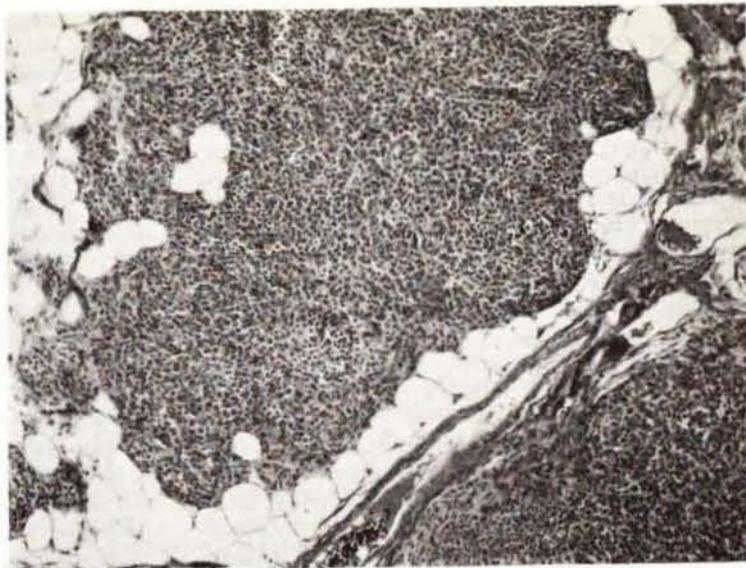


Figure 5-7 The hypoplastic thymus lacking lymphoid elements and Hassall's corpuscle is a salient feature of autosomal recessive combined immunodeficiency disease. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)

Figure 5-8 Thymus in ataxia-telangiectasia. Severe lymphoid depletion in both cortex and medulla. Hassall's corpuscles are absent. (Courtesy of Dr. Robert A. Good, Director of Sloan-Kettering Institute, New York.)



The Wiskott-Aldrich syndrome is a sex-linked disease characterized by a central thrombopenia, eczema, and marked susceptibility to many infections. Patients with this syndrome lack the normal isoheamagglutinins and hemolysins and fail to produce antibodies to polysaccharide antigens. By contrast, the humoral antibody response to antigens such as tetanus toxoid is normal. In these patients a progressive deficiency of cell-mediated immunity develops. Like the patients with ataxia-telangiectasia, a high incidence of lymphoreticular malignant conditions is found. These malignant conditions may rapidly become widespread and seem to have great propensity to invade the central nervous system. It appears that the basic immunologic defect in these patients involves the afferent limb of the immune response and perhaps resides primarily in the macrophage processing of antigen.

Patients with a benign spindle cell tumor of the thymus have been described as having hypogammaglobulinemia, antibody deficiency syndrome, defects of cellular immunity, and often an absence of eosinophils. As with other hypogammaglobulinemic patients, they are prone to develop arthritis and other autoallergic and mesenchymal diseases.

The group of variable immunodeficiency disorders includes many syndromes which are not well understood. Included in this group are cases previously classified as "congenital" nonsex-linked or sporadic hypogammaglobulinemia, primary "dysgammaglobulinemia" of both childhood and adult life, and "acquired" primary hypogammaglobulinemia.

Treatment of Immunodeficiency

Considerable improvement is achieved in patients with B cell deficiency by replacement therapy with gamma globulins. Special precautions should be taken to avoid infection by hepatitis virus which frequently causes lethal liver disease in these patients. Some patients with thymic hypoplasia have been successfully treated with fetal thymus transplants. Although these transplants came from histoincompatible donors, they were able to reconstitute T cell function for a considerable time. Combined immunodeficiency disease can be corrected by transplanting a source of lymphoid stem cells, usually the bone marrow. The development of graft-versus-host disease is a serious complication, because the bone marrow gives rise to a population of immunocompetent lymphoid cells which make an immune response against the histoincompatible and immunologically incompetent host. Identity across the HL-A histocompatibility locus is generally required for the successful acceptance of these bone marrow transplants.

Disorders of Immunoglobulin Metabolism

These may occur secondarily to a variety of pathophysiologic mechanisms. As has been discussed above, hypogammaglobulinemia may be due to a primary immunodeficiency of the B lymphocyte system affecting one or more classes of immunoglobulins. Alternatively, hypogammaglobulinemia may be secondary to excessive loss of immunoglobulins into the urinary or gastrointestinal tracts. Finally, abnormal im-

munoglobulin levels may be secondary to disorders of endogenous immunoglobulin catabolic pathways. Accelerated catabolism may affect one or more immunoglobulin classes.

Accelerated catabolism of IgG has been observed in patients with myotonic dystrophy. This autosomal dominant disorder is characterized by myotonia, muscle wasting, premature baldness and testicular atrophy, cataracts, and electrocardiographic abnormalities. The serum levels and metabolism of albumin, IgM, IgA, IgD, and IgE are normal. In contrast, serum levels of IgG are markedly reduced as the result of an accelerated catabolism.

An increased rate of catabolism of IgA has been observed in patients with isolated IgA deficiency or ataxia-telangiectasia, when they produce anti-IgA antibodies.

Alternatively, generalized hypercatabolism of many serum proteins, including immunoglobulins, is often present in patients with Wiskott-Aldrich syndrome or with familial hypercatabolic hypoproteinemia. Elevated urinary or serum levels of immunoglobulin light chains are observed in patients with tubular proteinuria or uremia when the normal site of catabolism of these molecules, the proximal convoluted tubules, is damaged.

Disorders of the Complement System

A variety of genetically determined abnormalities of the complement system have been described. These can be classified as (1) defects in inhibitors which result in the spontaneous consumption of complement components, (2) synthesis of defective complement molecules, and (3) absence of a complement component.

Hereditary angioedema is caused by a defective synthesis of C1 esterase inhibitor. In normal persons, C1 esterase inhibitor blocks the activity of C1s and is therefore capable of suppressing the progression of the complement sequence beyond C1. This enzyme also inhibits the activity of certain enzymes active in the clotting system, including the Hageman factor, plasmin, and prekallikrein. Patients with hereditary angioedema suffer from episodic triggerings of the complement system and develop severe angioedematous reactions in various tissues, particularly those of the upper respiratory tract. It is unclear why these patients have episodic disease, but trauma, menstruation, and infections may precipitate clinical crises. This disease may be observed at any age but usually first manifests itself during late adolescence or early adult life. The recurrent attacks of edema are mostly confined to skin, gastrointestinal tract, and respiratory mucosa. The edema is nonpitting and nonpruritic. The episodes last for about 48 to 72 hours. The involve-

ment of the larynx is most serious and many patients may die from suffocation. Hereditary angioedema exists in two genetic variants. In one type no C1 esterase is produced and in the other the protein is made but is not functional.

Patients with a defect in C3 catabolism as the result of an abnormal activity of C3-proactivator suffer from frequent recurrent pyogenic infections. Familial C5 dysfunction has been described in patients with Leiner's syndrome who suffer from generalized seborrheic dermatitis, intractable severe diarrhea, recurrent local and systemic bacterial infections usually of gram-negative etiology, and a marked wasting and dystrophy. Deficient opsonic activity of the patient's serum with yeast cells is the diagnostic laboratory finding in this disease.

Autosomal recessive C2 deficiency is usually not associated with any clinical disease. Although the sera of these patients are extremely defective in hemolytic activity, many biological functions of the complement system, like immunoadherence and facilitation of phagocytosis, seem unimpaired. It appears that C2 deficient individuals have an unusually high incidence of hypersensitivity disease.

Patients with the autosomal recessive form of combined immunodeficiency disease have extremely low serum levels of C1q. Since C1q has an electrophoretic mobility of a gamma globulin, it is particularly interesting that this protein should be defective in patients lacking both B and T lymphoid cells. In contrast, patients with sex-linked, recessive combined immunodeficiency as well as the various forms of hypogammaglobulinemia show only slight to moderate reduction of C1q levels.

Experimental strains of mice and rabbits have been described which lack C5 and C6, respectively. Low serum levels of complement can also be observed secondary to a variety of diseases, particularly in immune complex diseases such as acute glomerulonephritis, systemic lupus erythematosus, and poststreptococcal glomerulonephritis.

Secondary Immunodeficiency Diseases

Although primary immunodeficiencies have received considerable attention, these diseases are rather uncommon. In contrast, depressed immune function occurs quite frequently as a consequence of various disease states, especially those that cause disturbances in general metabolism and which induce stress. These secondary immunodeficiencies are frequently observed in certain infections, malignant conditions (especially of the lymphoid system), excessive loss of proteins or cells from the body, drug therapy, debilitating diseases, and aging. Clinically, im-

munodeficiency is first indicated by more frequent and persisting infections with one specific microorganism or a variety of microorganisms. Secondary immunodeficiencies may affect both humoral and cellular immune systems, and may operate at the induction (afferent limb) or expression (efferent limb) of immunity. The methods of evaluation of humoral and cellular immune function in patients with suspected secondary immunodeficiency are listed in Tables 5-1 and 5-2.

Certain infections may lead to decreased immune function. For example, secondary immunodeficiency is usually present in infants with congenital rubella syndrome. Infection with rubella virus is contracted during the second and third month of gestation. These patients have a continuing active viral infection from birth up to 18 months of age and produce antiviral antibodies, primarily of the IgM type. Increased serum IgM levels are frequently accompanied by markedly reduced IgG concentrations; occasionally there is a generalized hypogammaglobulinemia. During active rubella infection, peripheral blood lymphocytes may fail to undergo phytohemagglutinin-induced blastogenesis. Infections with measles (rubeola) virus frequently induce a temporary loss of delayed hypersensitivity. Tuberculin-positive patients may temporarily lose their ability to express a delayed allergic reaction to tuberculin protein during a measles infection. Lymphocytes from these patients are unable to respond with blastogenesis to antigen, although their responsiveness to phytohemagglutinin or other mitogens is unaltered. Lymphocytes infected by *Mycoplasma* also have diminished ability to respond to antigenic and mitogenic stimulation.

Most immunoproliferative diseases are accompanied by a secondary immunodeficiency state. Abnormal proliferation may affect the B and T cell immune systems. Best known are the secreting B cell tumors, which include multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease. These disorders have often been called the monoclonal gammopathies because these plasma cell tumors produce excessive amounts of an immunoglobulin of sharply restricted molecular heterogeneity. These paraproteins may be of any one of the immunoglobulin classes and may have the same basic molecular structure as their normal counterparts. An exception is a paraprotein of heavy chain disease which lacks a portion of the Fd fragment of the heavy chain (see p. 100). Since numerous myeloma proteins have been found to have antibody specificity for certain antigens, it is currently believed that all myeloma proteins are antibodies of restricted molecular specificity. Light chains of either kappa or lambda types may be produced in excess of heavy chains and may circulate as free dimers

of homogeneous light chains. Because of their small size they are rapidly excreted in the urine (Bence Jones proteins).

Patients with multiple myeloma often suffer from recurrent infections with *Pneumococcus* and other highly pathogenic bacteria. They have no unusual susceptibility to infections with staphylococci, enterobacteria, or fungi. Severe deficiency of the B cell immune system can be demonstrated by the low levels of serum immunoglobulin and seems to be a result of decreased synthesis of normal immunoglobulin molecules. Antibody synthesis against exogenously administered antigen is markedly deficient. In contrast, cellular immune function is grossly normal.

Patients with Waldenström's macroglobulinemia have excessively high serum levels of a high molecular weight protein which has the chemical and immunochemical characteristics of IgM. Because of the high intrinsic viscosity of IgM protein, these patients have increased viscosity of the serum, which leads to sluggish blood flow, thrombosis, central nervous system disturbances, and bleeding. Most commonly affected are the skin, nasal mucosa, and gastrointestinal tract. Sluggish blood flow of the retinal vessels may eventually cause thrombosis of retinal veins and blindness. Frequently, the patient's red cells, granulocytes, and particularly platelets may be coated with macroglobulins, which results in impaired survival and function of these elements. Lymphadenopathy and hepatosplenomegaly are salient features. Hyperproduction of IgM macroglobulin is often accompanied by decreased synthesis of other immunoglobulins. Many patients produce detectable amounts of monomeric, low molecular weight macroglobulins (7S IgM). Approximately 10 per cent have demonstrable Bence Jones proteinuria.

The recent elucidation of the structure of the immunoglobulins (see p. 101) and the detailed characterization of the proteins in serum and urine of patients with proliferative disorders of plasma cells and lymphocytes have led to a better understanding of the biosynthetic alterations accompanying these disorders. These diseases are now frequently classified on the basis of the products of the neoplastic cells. Multiple myeloma is defined by the type of immunoglobulins produced (G, A, D, and E myeloma), and the biosynthetic processes in myeloma and macroglobulinemia can be either the balanced synthesis of heavy and light chains, resulting in homogeneous serum immunoglobulin, or the unbalanced synthesis manifested by the presence of light and occasionally heavy chains as an additional, or at times, the sole detectable product. In addition, the recognition of several new disorders of immunoglobulin synthesis has resulted in the identification of a number of new syndromes. Best described among

these are the group of heavy chain diseases which represent examples of abnormal immunoglobulin synthesis and assembly. Three types of heavy chain diseases corresponding to the heavy chains of IgG (gamma), IgA (alpha), and IgM (mu) are now recognized. Gamma chain disease is most frequently seen in the middle aged and elderly. A rather characteristic clinical picture which resembles a malignant lymphoma rather than multiple myeloma includes lymphadenopathy and hepatosplenomegaly. Involvement of the nodes of Waldeyer's ring resulting in palatal edema is particularly striking. Almost all patients suffer from frequent episodes of infection. Anemia, leukopenia, eosinophilia, and hyperuricemia are common but are relatively nonspecific laboratory findings. Definitive diagnosis of gamma chain disease can only be made by immunochemical analysis of serum and urine proteins. Characteristically, there is a broad peak with a mobility of a beta globulin present in both serum and urine. Amino acid sequence analysis of gamma chain disease proteins has demonstrated deletion of most of the Fd fragment as a consequence of loss of interchain disulfide bridges. Alpha chain disease has been found in patients with malignant lymphoma involving the small intestine and is usually associated with severe malabsorption. A few cases of mu chain disease have been described in patients with chronic lymphocytic leukemia.

Patients with Hodgkin's disease develop a progressive immunodeficiency of the cellular immune system while humoral immune function appears essentially normal. Both the afferent and efferent limbs of T cell-mediated cellular immunity are deficient. Although patients with Hodgkin's disease have a normal resistance to infections with *Pneumococcus*, *Haemophilus*, and *Streptococcus*, they are susceptible to tuberculosis and fungal infections.

Patients with chronic lymphocytic leukemia develop deficiencies of both the T and B cell immune systems. Both immune systems are essentially normal in acute lymphocytic leukemia. In later phases of these lymphoreticular malignant conditions, apparently as a consequence of continuous anticancer chemotherapy, defective host defense mechanisms allow recurrent infections to become an immense problem.

The lymphoreticular malignant conditions, like the lymphocytic leukemias, the lymphomas, reticulum cell sarcomas, and Hodgkin's disease, have thus far been classified on the basis of morphologic and clinical observations. However, with respect to our rapidly expanding knowledge of the immune lymphoid system, it is advisable to classify these proliferative disorders according to functional characteristics of B and T lymphocytes. Experimental evidence indicates that chronic lymphocytic leukemia is probably a non-

secreting B cell leukemia and certain forms of malignant lymphoma are T cell tumors.

Patients with nephrotic syndrome have low serum levels of albumin and gamma globulin because the excessive amounts of these proteins are lost in the urine. Consequently, these patients are very susceptible to infections by encapsulated microorganisms as well as gram-negative bacteria. Nephrotic patients respond well to immunogenic stimuli but specific antibody is rapidly lost in the urine. The most affected immunoglobulin is IgG, while IgM and the beta and alpha globulins of the complement system are normal. This is also the case in classic lipoid nephrosis of children. In contrast, patients with acute glomerulonephritis and certain forms of chronic glomerulonephritis not only lose protein in the urine but also deposit immune complexes and complement in their kidneys. These patients may have complement deficiencies because of an excessive rate of utilization. Some patients with hypocomplementemic forms of chronic renal disease may have a decreased rate of synthesis of complement components, especially C3. These patients have a diminished ability to develop inflammatory responses.

Protein losses from the intestinal tract during certain acute and chronic diseases of the bowel may lead to hypogammaglobulinemia. In intestinal lymphangiectasia, both serum proteins and lymphocytes are lost in the stool. Such patients have low immunoglobulin levels, lymphopenia, and deficient immune function of both B and T cell systems.

Patients who have sustained major burn injuries develop immunodeficiencies. A transient hypogammaglobulinemia may occur during the first week because of losses through the burn lesions. Increased synthesis of immunoglobulins overcompensates for these losses one week later. Lymphocyte depletion occurs as a result of destruction of lymphocytes under the influence of a stress-induced increase of adrenocorticosteroid production. The markedly decreased resistance of burn patients to infections is mainly due to the relative inability of their neutrophils to kill phagocytosed bacteria.

TUMOR IMMUNOLOGY

Tumor-Associated Antigens (Tumor-Specific Antigens)

The majority of tumors, including those induced by chemical carcinogens and viruses, as well as spontaneously arising tumors, carry antigens which elicit immune responses in the host. Tumors induced by chemical or physical agents express individually distinct antigens so that cross-immunization is rarely possible even in

tumors of similar morphology induced by the same carcinogen. In contrast, virus-induced tumors contain cross-reacting antigens showing virus-related specificities. Immunization against one tumor may lead to protection against other tumors induced by the same virus. This difference in the specificities of antigens associated with tumors induced by chemical carcinogens and oncogenic viruses has provided a method for determining the etiology of spontaneous tumors. Since several virus-induced tumors have recently been shown to have individually specific antigens besides the common antigens coded for by the virus, this distinction is less clear and definition of the antigenic tumor specificities does not provide conclusive evidence of its etiology. In addition, several chemical carcinogen-induced tumors stimulate the production of antibodies which cross-react with antigens associated with certain viruses.

There are two major categories of tumor-associated antigens in virus-induced neoplasms: (1) the virus-specific antigens and (2) the newly formed antigens, i.e., the antigens that were not present in the normal cells before neoplastic transformation, or on the virions. Antigens belonging to the latter category may arise as (a) products of a specific interaction of the viral genome with the host genome, (b) a specific uncovering of normal or preexisting (embryonic?) cell products, or (c) as a consequence of derepression of intracellular type-C RNA viruses.

The virus-specific antigens may be structural components of the virion or virus-coated products synthesized by the neoplastic cells. Although viral antigens are frequently demonstrable in RNA virus-induced neoplasms, they are rarely seen in tumors induced by DNA viruses. In leukemias induced by RNA viruses, cell surface antigens may be shared with those of the virion. The viruses mature at the cell membrane by budding and the virus particles which are continuously shed by the leukemic cells receive an outer coat from the cell membrane.

Tumor-specific antigens in DNA virus-induced neoplasias are not identical with the antigens of the mature virion and are present even when infectious virus production by the transformed cells cannot be demonstrated. Since these antigens relate specifically to the inducing virus, their presence and permanence suggest that viruses leave at least part of their genetic information in a cell after neoplastic transformation. In view of the cross-reactivity of cells of different types and between tumors in different species, it is likely that the new antigens on the surface of DNA virus-induced tumor cells are determined by the viral genome. On the other hand, tumors induced by different viruses do not cross-react, even when these tumors are morphologically similar and are present in the same species. Exceptions to these

rules for cross-reactivity of chemical- or virus-induced neoplasms may occur when the transformed cells express new antigens related to derepression of certain host genomes or of intracellular virogenes.

Derepression of cell genome-coated products occurs in certain types of tumors giving rise to tumor-associated antigens which cross-react with antigens found in embryonal tissue but not in adult host tissue. Most frequently described are the carcinoembryonic antigen of carcinoma of the colon and the alpha fetoprotein associated with hepatic malignant disease in man. The relation between the appearance of these embryonal antigens and neoplastic transformation is not clear, since carcinoembryonic antigen has recently been found in colonic and other gastrointestinal tissue in patients with various non-neoplastic disorders. Several experimentally induced tumors also express embryonal tissue antigens.

Recent studies support the concept that vertebrates contain the genetic information for producing type-C RNA tumor virus in an unexpressed form in their somatic cells as well as in their germ cells. The viral oncogene theory proposes that the endogenous virogenes (the genes for production of type-C viruses) and the oncogenes (that portion of the virogene responsible for transforming a normal cell into a neoplastic cell) are maintained in an unexpressed form by repressors in normal cells. Various agents, including radiation, chemical carcinogens, and exogenously added viruses, may transform cells by "switching on" the endogenous oncogene information. It is under these conditions that the major internal antigen of type-C virus, the group specific or gs antigen can be detected in tumorous tissue.

One approach to studying the etiology of human malignant disease has been the detection of immunologic cross-reactivity in a variety of human tumors. Since all tumors induced by the same virus contain common tumor-associated antigens, while neoplasms induced by different viruses or by nonviral carcinogens usually lack a common antigenicity, one would anticipate a common viral etiology from neoplasms possessing common tumor-associated antigens. However, immunologic cross-reactivity between tumors can be due to the presence of embryonic and gs antigens or antigens induced by virus infection of tumor cells. Patterns of cross-reactivity have been demonstrated between antigens associated with a variety of tumors. Various human cancers originating in the same organs appear to share antigens recognized as foreign by the immune system of the patient while being undetectable in normal cells of the same organ. Certain human tumors share common antigens not found on other tumors. They include neuroblastoma, mammary carcinoma, endometrial cancer, colonic car-

cinoma, bladder tumors, and malignant melanomas. These observations suggest a viral etiology for the majority of human neoplasms, provided that one postulates that there is a large array of viruses, each of which is associated with neoplasms of a certain origin. This could be the case for RNA viruses, which are usually specific for the type of tumor and organ affected. Unfortunately, there are no data to support the hypothesis that these human tumors are induced by tumor viruses. On the other hand, there is evidence that common antigens detected in Burkitt's lymphoma and nasopharyngeal tumor are associated with the presence of Epstein-Barr (EB) herpes type virus in these neoplasms. The question to be resolved for these neoplasms is whether they carry EB virus as an etiologic agent or as a passenger virus.

Demonstration of Tumor Immunity

It is generally accepted that both cell-mediated and humoral immune response play a major role in host-tumor relationships. Histologic evidence of tumor immunity is indicated by the infiltration of several types of tumor by mononuclear lymphoid cells, which is frequently accompanied by increased cellularity of the regional lymph nodes. For instance, in malignant melanoma, this appearance is most frequently found around primary tumors and early tumors. The lymphocellular response usually decreases as time elapses and the tumor grows. Increased proliferation of mononuclear cells in the regional lymph nodes and infiltration of lymphocytes and plasma cells around the periphery of the tumor have often been associated with a favorable prognosis of patients with various types of malignant disease (e.g., mammary carcinoma, gastric carcinoma, malignant melanoma, testicular seminoma, and urinary bladder tumor).

Several in-vitro tests have been developed to analyze humoral and cellular immunity to tumor cells. Humoral antibodies can be demonstrated by tests for cytotoxicity, membrane immunofluorescence, and mixed hemadsorption and immune adherence. Cytotoxicity tests determine tumor-specific antibodies which, in the presence of complement, exert a cytolytic effect on tumor cells. Although these cytotoxicity tests may be useful in determining humoral immunity to lymphoma and leukemia cells, they rarely work for sarcomas and carcinomas. Cell surface antigens are demonstrable by membrane immunofluorescence in Burkitt's lymphoma and nasopharyngeal carcinoma in which cross-reacting antigens associated with the EB virus can be detected.

Cell-mediated immunity to malignant disease has been demonstrated by use of in-vitro assays with tumors from experimental animals as well

as in man. These tests are based on the fact that tumor-specific immune lymphocytes, upon interaction with tumor cells, can be activated to produce soluble factors, lymphokines, which participate in the amplification system of cell-mediated immunity and which may lead to destruction of the tumor cell. The major evidence for cell-mediated immune reactions against human tumors has come from studies performed with the colony-inhibition technique. Tumor cells are first grown in vitro and are then exposed to tumor-specific immune lymphocytes. The inhibitory effect of these lymphocytes is assessed by a reduction in the number of tumor colonies as compared to the effect with nonimmune lymphocytes.

The first human tumor to be studied by the colony-inhibition technique was neuroblastoma. It was found that peripheral blood lymphocytes from patients with such tumors inhibited colony formation of neuroblastoma cells, whether they were of autochthonous or allogeneic origin. Normal skin fibroblasts from tumor donors were not inhibited. Lymphocytes from patients with tumors other than neuroblastomas and from donors not having cancer did not inhibit these neuroblastoma cells. Thus, the lymphocyte effect was specific and could not be attributed to immunity to normal alloantigens. These observations also implied that neuroblastomas have a common tumor-associated antigen.

Similar results have been obtained with the colony-inhibition test in a large variety of human malignant conditions (Table 5-4). In many in-

TABLE 5-4 ANTIGENIC CROSS-REACTIVITY BETWEEN TUMORS OF THE SAME TYPE

Tumor Type	Number of Patient-Tumor Cell Combinations Positive Per Number of Combinations Tested*
Melanoma	41/41
Colon cancer	25/31
Breast cancer	12/14
Lung cancer	5/6
Sarcoma	2/3
Endometrial cancer	2/3
Ovarian cancer	3/5
Testicular tumor	8/10
Squamous cell cancer	2/2
Kidney cancer	1/1
Wilms' tumor	1/2
Parotid tumor	1/1
Total	103/119 (87%)

*Tumor patient lymphocytes were tested on target tumor cells derived from different donors all having the same type of neoplasm. Information courtesy of Dr. K. E. Hellström.

stances, the specific inhibition of colony formation by lymphocytes from patients with a certain type of tumor has provided evidence of antigenic cross-reactivity within (but not between) groups of tumors of the same morphology.

Several neoplasms in experimental animals (e.g., Shope papilloma in rabbits and Moloney virus-induced sarcomas in mice) and man (e.g., neuroblastoma) undergo spontaneous regression. This was not due to alteration of cellular immunity to the tumor cells, since lymphocytes from both persistors (in whom tumors were growing) and regressors (in whom the tumors had regressed) had the same inhibitory effect on colony formation. On the other hand, incubation of the tumor cell cultures with serum from persistors abolished the tumor-inhibiting effect of both persistor and regressor lymphocytes (Fig. 5-9). Serum from regressors did not interfere with the antitumor activity of these lymphocytes.

The serum of tumor-bearing individuals has often been shown to block the activity of tumor-specific immune lymphocytes in the colony-inhibition technique. This serum blocking activity has been associated with tumor-specific antibodies or with antigen-antibody complexes. Pro-

vided that these antibodies or complexes have a similar effect *in vivo* as *in vitro*, the presence of serum blocking activity would be a powerful mechanism of escape from an efficient lymphocyte-mediated immune surveillance of tumor growth. If the development of blocking antibodies plays an important role in enabling tumors to grow in an immunologically sensitized host, it should be possible to inhibit tumor growth by measures that counteract the blocking activity, either by the prevention of blocking antibody formation or by intervention with its action. In this respect, it was found that in Moloney sarcomas of mice and other tumor systems admixture of regressor serum to persistor serum abolishes the blocking activity of regressor serum, and the blocking activity of persistor serum is not well understood.

Host-Tumor Relationships and Immunosurveillance

From a teleologic standpoint, it is generally accepted that the lymphoid system may have evolved in vertebrates for the purpose of seeking out, recognizing, and destroying malignantly al-

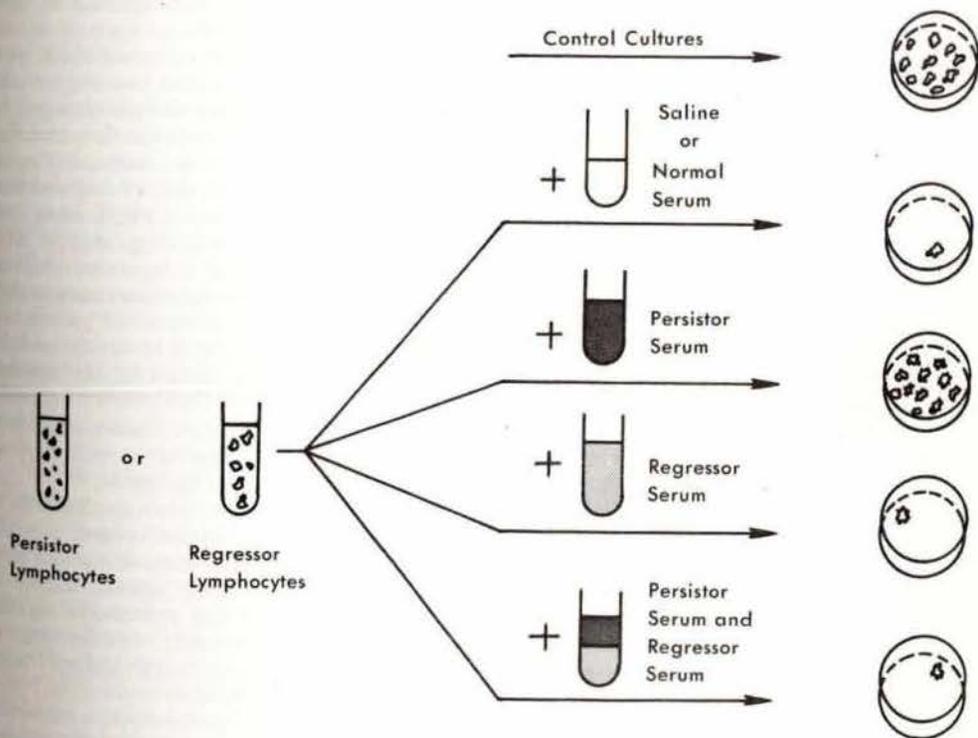


Figure 5-9 Colony-inhibition assay. Lymphocytes from hosts with growing tumors (persistors) and from hosts with regressed tumors (regressors) inhibit the *in-vitro* formation of tumor colonies. Serum from persistors block the tumor-inhibiting effect of both persistor and regressor lymphocytes. In contrast, serum from regressors does not exhibit this blocking effect and may even have the capability to "unblock" the blocking activity of persistor serum.

TABLE 5-5 MALIGNANT CONDITIONS IN PATIENTS WITH IMMUNODEFICIENCY*

Primary Disease	Number of Patients Who Developed Malignant Condition	Types of Malignant Conditions	Percentage of Patients With Cancer
Infantile sex-linked agammaglobulinemia	5	Leukemia	5 to 10 per cent
Ataxia-telangiectasia	42	Many	10 to 15 per cent
Wiskott-Aldrich syndrome	13	Mostly lymphoreticular	> 10 per cent
Common variable immunodeficiency	30+	Many	5 to 10 per cent
Severe combined immunodeficiency	3	--	1 to 10 per cent

*Observations by Dr. R. A. Good.

tered tissue. This surveillance system may be just another homeostatic control mechanism through which the host maintains its integrity. Faulty immunosurveillance is generally associated with increased incidence of malignant disease. Decreased immune function normally present in immature or aging animals or experimentally induced by irradiation, neonatal thymectomy, and administration of immunosuppressive drugs or antilymphocyte serum is often associated with an increased susceptibility to malignancy. In addition, much clinical evidence links the lymphoid system inextricably with malignant change in man. Regardless of the nature of the disorder, all forms of primary immunodeficiency of man have shown evidence of increased susceptibility to malignant disease. For example, patients with a sex-linked recessive Bruton type agammaglobulinemia are inordinately susceptible to the development of leukemia. A high incidence of malignant disease is also found in ataxia-telangiectasia. These diseases are frequently reticulum cell sarcomas, lymphosarcomas, and leukemias, but epithelial malignant diseases, especially of the gastrointestinal tract and of other supporting and mesenchymal tissues, have also been reported. Patients with the Wiskott-Aldrich syndrome have a high frequency of malignant disease, the most common form of which is a reticulum cell tumor which often affects the brain as well as the lymphoid and hematopoietic organs. A high incidence of malignant disease is encountered in most other immunodeficiency syndromes (Table 5-5).

Conversely, malignant disease of the lymphoid system such as Hodgkin's disease, multiple myeloma, and chronic lymphatic leukemia all manifest severe malfunction of humoral or cellular immunity, or both. Such deficiencies have also been described in patients with malignant diseases of

nonlymphoid organs. These patients respond subnormally to immunogenic stimulation with tetanus toxoid, dinitrochlorobenzene (DNCB), and tuberculin. For instance, the inability of breast cancer patients to become sensitized to DNCB has been associated with a poor prognosis. It is quite likely that the immune defect in these cancer patients is a consequence of advanced disease and not its cause.

The concept of immunosurveillance should by no means be construed as the only mechanism serving to control neoplastic change. For instance, the role of hormones in the surveillance of endocrine tissue and their tumors is well documented. Contact inhibition of isolated aberrant cells provides another mechanism of surveillance. Closely resembling contact inhibition is the phenomenon of allogeneic inhibition, by which cells with different structures on their cell surfaces mutually inhibit their growth when in contact with each other. A newly formed neoplastic cell may be eliminated by the normal cells around it long before it becomes recognized by the immune system. The significance of these nonimmunologic mechanisms of surveillance against malignancy is difficult to assess.

The combined surveillance mechanisms appear to be most effective in the control of malignant change. In spite of the continuous and extensive exposure to carcinogenic agents, cancer is very uncommon in most age groups. In addition, we probably carry oncogenic viruses (e.g., type-C RNA viruses) which may induce neoplasms under certain conditions.

Although the association between immunodeficiency and the increased incidence of malignant disease is well documented, most cancer patients have no history of apparent immunodeficiency disorders. It is in these instances that the relation between immunologic aberrations

tion and neoplastic growth is poorly understood. Several mechanisms have been proposed by which tumors can overcome immunosurveillance by the lymphoid system. The escape of neoplastic growth from immunosurveillance may occur at the level of induction of the immune response (afferent limb) or at the level of expression of immunity (efferent limb).

In the afferent limb of the immune response, the tumor antigens should be regarded as tumor immunogens. The structure and physicochemical nature of these immunogens, as well as the amounts present in the tumor and available for immunogenic stimulation, obviously have great influence on the outcome of the tumor-specific immune response. When a cell undergoes a neoplastic change, it seems quite likely that sufficient amounts of tumor-specific antigen must be present on that cell to stimulate the immune system. Therefore, this neoplastic cell will probably have to undergo a number of divisions before sufficient quantities of tumor-specific antigens are present to elicit an immune response. Immunogenic stimulation of the lymphoid system is probably induced by the antigenic structures present on tumor cell break-down products, such as cell membranes, which enter the regional lymph nodes by way of the draining lymphatics. When the immunogenicity of the tumor is low, or when the tumor has a high cellular proliferation rate, the tumor may outgrow its susceptibility to the effects of the immune system and thus escape immune surveillance.

In experimental studies, it has been shown that exposure of the animals to an oncogenic virus or a carcinogen will temporarily suppress the immune response. With reference to the unknown etiology of most human cancers, it is not known whether this potential suppression of the immune response is relevant to the development of human malignant disease.

Immunologic factors may suppress those cells with the most surface immunogenicity and "select" those cells with the least immunogenicity. This immunoselection may represent a mechanism by which a neoplastic cell with low immunogenicity "sneaks through" the immunosurveillance of the host.

The genetic background of the host may also influence his immune system. It has been demonstrated that the quantity as well as the quality of the immune response is controlled by "immune response genes," some of which appear to be associated with transplantation genes. Several investigators have observed that these operate at the level of the antigen-reactive thymus-derived lymphocyte, but others have indicated that antibody-producing B cells may also be involved. Evidence suggests that these genes may be one of several genetic factors affecting a variety of neoplastic and autoallergic diseases. For instance,

the resistance of mice to Gross virus leukemogenesis is under the influence of two genes, one of which is the immune response gene, the other a histocompatibility gene. In this respect it is interesting to note that certain types of malignant disease occur in higher frequency in patients having specific combinations of transplantation antigens. For instance, the presence of HLA-5 antigen is associated with an increased incidence of Hodgkin's disease.

It is possible that the host has developed immunologic tolerance to his tumor, similar to the chimeric state seen after transplantation of hematopoietic and other tissues, so that rejection does not occur. Pregnancy represents a comparable balanced immunologic relationship whereby the graft, the fetus, survives in an immunologically incompatible host, the mother. In chimerism, as in the host-tumor relationship, we appear to be dealing with a complex set of biological problems involving the potentiality for adaptation by the graft or tumor and the host. Our attention in tumor immunology has been focused on immunologic adaptation by the host. Attention must be given to the possible contribution of the tumors in this process.

Even when a host develops a vigorous immune response against the tumor, i.e., an effective cellular immunity which may be accompanied by humoral cytotoxic antibody formation, the possibility still exists that the tumor survives in the host. This situation is prevalent in many tumor systems as demonstrated by the presence of blocking factors which interfere with the anti-tumor activity of tumor-specific immune lymphocytes in the colony-inhibition technique. These blocking factors resemble the antibody molecules responsible for the phenomenon of immunoenhancement. Experimental studies have shown that passive transfer of tumor-specific antibodies into a syngeneic host exerts an enhancing effect on the growth of a transplant of that tumor. Also, mixing of humoral antibody with tumor cells *in vitro* made possible their development into tumors under conditions which otherwise precluded such events. Several explanations have been proposed about the mechanism of immunoenhancement by humoral antibodies. An essential component is that such protective action by humoral antibodies is likely to take place in situations in which the host defense is primarily mediated by immune lymphocytes. One explanation is that immunoenhancement is active at the efferent limb of the immune response. The antigenic determinants on the tumor cell surface are blocked by antibody so that immune lymphocytes do not recognize these antigens and therefore do not attack tumor cells. These humoral antibodies may also prevent tumor antigens from sensitizing lymphocytes or they may interfere with proliferation of new reactive popu-

lations of immune lymphocytes. These postulates for the mechanism of action of enhancing antibodies may also apply to those factors which block the activity of tumor-immune lymphocytes in the colony-inhibition assay. However, recent evidence seems to indicate that the blocking factors are circulating complexes between antibody and tumor-associated antigens.

An important consideration should be given to the role of tumor-associated antigens in the efferent limb of the host immune response to the tumor. They can be classified into two groups: (1) those antigens present on the cell surface that have been called tumor-rejection antigens, and (2) the soluble antigens localized in the cell cytoplasm and nucleus to which specific immunity has little effect on tumor growth. The availability of tumor-rejection antigens to the effector mechanisms of humoral and cellular immunity greatly influences their efficiency. For instance, tumor antigens deeply embedded in the cell surface are inaccessible to antibodies as well as to immune lymphocytes. Also, the density of tumor-rejection antigens on the cell surface is relevant to the effectiveness of certain types of immune responses. For example, IgG complement-fixing antibody requires a relatively high density of tumor antigen to induce a cytotoxic effect on the tumor cell because of the requirement of the close proximity of at least two IgG molecules on the surface to activate the complement sequence (see Chapter 4). The same cytotoxic complement-fixing antibody does not exhibit this effect when the density of the tumor-rejection antigen is low. In this situation, by combining with the antigens on the tumor cell, this antibody may interfere with the effector mechanisms of tumor-specific cellular immunity.

Clinical Applications of Tumor Immunobiology

Our rapidly expanding knowledge of tumor immunology has provided a basis for the immunologic approach in the diagnosis and treatment of malignant disease. Since the demonstration of tumor-specific antigens in a great variety of human malignant diseases, many attempts are underway to isolate and purify these substances. The development of practical tests for tumor antigen detection should enhance diagnosis and permit detection of residual tumor after therapy.

Tumor-specific immunity, both cell-mediated and humoral, can now be assessed *in vitro* on an experimental basis. However, most of the methods involved are technically difficult and not practical for routine use.

Evaluation of the general status of the immune system of the tumor patient can be of prognostic value. For example, total lymphocyte counts are low, delayed hypersensitivity reactions to com-

mon antigens are less frequent, phytohemagglutinin-induced transformation of lymphocytes is reduced, and susceptibility to sensitization to DNCB is impaired in cancer patients who have extensive and disseminated disease. Unfortunately, these observations are applicable to some tumors but not to others.

Another important application of tumor immunology is the use of immunologic principles in the treatment of cancer. In most cases the central problem of tumor immunotherapy is not that of inducing the patient to become immune to his tumor, but rather to make existing immunity more effective in controlling tumor growth. Immunotherapy must attempt to direct the interaction between tumor growth and tumor immunity in favor of the host, either by quantitative augmentation of immunity or by qualitative alteration of the immune response so as to expose new tumor vulnerabilities. The approach of augmenting tumor-specific cell-mediated immune mechanisms or depression of the humoral immune response should be considered with some skepticism, since many of the immunologic aspects of the interaction between host and tumor are still poorly understood.

Attempts to increase cell-mediated immunity by adoptive transfer of allogeneic leukocytes from other individuals who had spontaneous regression or who had been tumor-immunized has had limited success. This may be attributed to the relatively short life span of the histoincompatible lymphocytes or to a graft-versus-host reaction occurring in patients receiving immunosuppression by their tumor or by chemotherapy. On the other hand, adoptive transfer through the use of transfer factor obtained from tumor-specific immune lymphocytes may be a potentially useful approach.

Another approach to augmentation of active tumor immunity is to increase or alter the immunogenicity of tumor cells. For example, tumor cells have been altered chemically or enzymatically so that they become more immunogenic. Painting of skin sensitizers such as DNCB on superficial squamous skin cancers of DNCB-sensitized patients, has induced selective tumor rejection. This method represents a new approach to the control of dermal cancers.

In addition to the specific augmentation methods mentioned above, nonspecific augmentation of tumor immunity appears to have been achieved through immunization with BCG. Clinical trials using this approach to immunotherapy of leukemia have been relatively successful.

Finally, cautious manipulation of the humoral immune response of the cancer patient (e.g., from blocking antibody to unblocking antibody) or administration of unblocking antibody with tumor specificity may offer a promising approach to immunotherapy.

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