The immunology of malignant disease

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Preface

This book is intended for clinicians concerned with the management of malignant disease. It is our hope that it will offer physicians a useful introduction to ideas and methods that will have increasing relevance to the investigation and treatment of cancer in man. It provides under one cover an assemblage of information about immune deficiency states associated with human cancer and does so against the background of similar knowledge available from the study of animal neoplasia and current concepts of immunobiology.

Drug therapy for human malignancy is effective in a number of clinical situations. Such chemotherapy is not, however, without risk. Impaired immunity is an inherent concomitant of many malignant disorders and may lead to infection. The immunosuppressive effect of cancer drugs may render cancer patients even more susceptible to infectious complications. These complications are considered and, where indicated, recommendations are made for prophylaxis and specific therapy.

The recognized immunosuppressive effect of anticancer drugs has led to their use in diseases where disordered immune function is suspected. The drugs have also found a place in the suppression of the allograft rejection reaction in human transplantation. The use of antitumor agents in both these areas is re-

viewed, and methods of clinical immunosuppression are detailed.

Definitive therapy is available for only a small fraction of human tumors; the treatment of most cancer is investigational. Much recent interest has focused on attempts to treat malignancy with a variety of immunotherapeutic approaches. These approaches are presented in the final section of this volume, but the theoretical bases for these approaches are dealt with throughout the book.

I am grateful to Dr. Joseph G. Sinkovics for his contribution of Chapter 2, "Immunology of Tumors in Experimental Animals." Dr. Sinkovics is also largely responsible for Chapter 5, "Tumors of Man."

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CHAPTER 1

Normal immune response in man and its reaction to malignant disease

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Table 1-1. *Physiologic immune mechanisms*

Nonspecific—innate immunity (polymorphonuclear leukocytes, monocytes, lymphocytes)	*Specific—adaptive immunity (lymphocytes, plasma cells)*
Inflammatory reactions	**Cell-mediated immunity**
Chemotaxis	Delayed hypersensitivity
Phagocytosis	Allograft rejection
Intracellular killing	
	Humoral immunity
Antimicrobial substances	Immunoglobulin formation (IgM, IgG, IgA, IgE, IgD)
Lysozyme	
Interferon	

Immunity in man originally defined a state free of contagion. The term is now a more compendious one and embraces a mosaic of physiologic mechanisms. They operate in unison to protect an intact human host from matter recognized as foreign. These mechanisms are listed in Table 1-1. Man has, for the most part, inherited from invertebrate ancestors his nonspecific immune capacities. The specific immune mechanisms are an evolutionary refinement. They are the common property of vertebrates from the lamprey on up the phylogenetic ladder to man. Taken together, the specific immune mechanisms constitute adaptive immunity.

This chapter will include a broad outline of the cellular events involved in the normal immune response. Most of the information is necessarily derived from animal experiments. Wherever possible, reference will be made to supporting studies in man. In immunobiology there is no generally agreed-upon pattern of cellular activity, and major questions remain to be answered. What is presented here represents a reasonable working hypothesis. It offers a useful framework on which to base the discussion of subsequent chapters.

SPECIFIC IMMUNE RESPONSES OF ADAPTIVE IMMUNITY

Lymphoid tissue in man is responsible for the specific immune mechanisms that make up adaptive immunity. Lymphoid tissue may be conveniently considered as either central or peripheral in type.[1] Central lymphoid tissue is that which originates directly from or lies in close association with intestinal epithelium. The chief example of central lymphoid tissue is the thymus, which develops from the third and fourth pharyngeal cleft pouches. Peripheral lymphoid tissue is found in the spleen and lymph nodes. The principal cell within peripheral lymphoid tissue is the small lymphocyte.

Two functionally distinct populations of small lymphocytes are recognized. Morphologically the two populations are indistinguishable. One cellular component, however, is dependent for its development on the thymus and effects the cell-mediated immune responses of delayed hypersensitivity and allograft rejection. It is referred to as *thymus-dependent* lymphoid tissue. The second distinct population of small lymphocytes responds to antigenic stimulation with the manufacture

of humoral antibody. It is *immunoglobulin-producing* lymphoid tissue. In the chicken, the development of cells of this type is influenced by the bursa of Fabricius, a central lymphoid organ associated with the hind gut. In man and other mammals a central lymphoid equivalent of the bursa of Fabricius has not yet been identified, but the possibility is strong that one exists.

The ideas advanced above are based on the theory that *antibody production (humoral immunity) and delayed hypersensitivity (cell-mediated immunity) are basically different immune processes.* There is good experimental evidence to suggest that this is so. A brief discussion of some of the experimental findings that support the two-component theory of the immune system will be presented at this point.

Antigenic stimulation will elicit delayed hypersensitivity or antibody formation or both types of reactions. In guinea pigs delayed hypersensitivity may be induced to benzene-o-sulfonic acid azo-guinea-pig albumin without antibody formation.[2] In man Type III pneumococcus capsular polysaccharide gives rise readily to humoral antibody without demonstrable delayed hypersensitivity. In the guinea pig, lymph nodes draining the site of immunization with polysaccharide (which gives only an antibody response) show a distinctly different histologic reaction from lymph nodes draining the site of immunization with a chemical agent (which will give initially only a delayed hypersensitivity response).[3] In the first instance, there is an increase in the size of lymphoid follicles with germinal center formation and proliferation of plasma cells in the medullary sinuses. In the second instance the lymphoid follicles remain unchanged, but there is intense mitotic activity among large pyroninophilic cells in paracortical areas. Some of these cells differentiate further to become small lymphocytes.

Comparable results were obtained in a study of allograft survival in mice.[4] Prior to graft rejection, large pyroninophilic cells were seen in the inner cortical region of draining lymph nodes. Germinal centers developed when the graft was practically destroyed. At about the same time plasma cells appeared in the lymph node medulla and isoantibody was measured for the first time in the serum of the grafted animals. It is of interest to note that in the mouse neonatal thymectomy will interfere with the ability to reject allografts and the development of a pyroninophilic cell response,[5] although germinal center formation, plasma cell development, and some humoral antibody formation remain relatively unaffected.

The argument for the existence of a separate cellular origin for humoral and cellular immunity is further supported by study of certain congenital and hereditary abnormalities in man. DiGeorge[6] has described a syndrome characterized by parathyroid and thymus gland aplasia. These glands are each derived from the third and fourth pharyngeal pouches, and a single congenital disturbance would understandably prevent the embryologic development of both. Affected infants show interesting immunologic abnormalities.[7] Humoral immunity is intact. There is, however, inability to develop delayed hypersensitivity to new antigens and to manifest the skin reactivity of established delayed hypersensitivity. Allograft rejection is greatly impaired. This congenital absence of the thymus is as-

sociated with a lack of cells in the subcortical (thymus-dependent[5]) areas of lymph nodes.

Cells migrating from the thymus tend to localize in this inner cortical region. The region has been noted in experimental animals to become depleted of cells by chronic drainage of a thoracic duct fistula, thymectomy, or administration of anti-lymphocyte serum. In these situations, as in the DiGeorge syndrome, germinal center activity in the superficial cortex and plasma cells in the lymph node medulla remain intact. Thymic allografts have been found to correct abnormalities of lymphocyte function in infants with DiGeorge syndrome.[8, 9] The transplanted thymus does not contribute immunocompetent cells to the recipient but seems rather to restore the immune capability of the recipient's own lymphocytes.[8]

In congenital agammaglobulinemia an almost completely reversed condition exists. There is depression of humoral immunity, but cell-mediated immunity is unimpaired. Delayed hypersensitivity responses and allograft rejection times are normal. Moreover, medullary plasma cells and cortical germinal centers appear histologically intact.

With the acceptance of the bipartite nature of adaptive immunity, we may now consider each of the two components that comprise the system.*

Humoral immunity—antibody formation (immunoglobulin-producing lymphoid tissue)

The humoral immune response to antigen consists of at least three phases. These merge, one into the other, but are sufficiently well defined to merit being distinguished from each other.[13] The time between antigenic challenge and the first appearance of circulating antibody is called the inductive or latent phase. There is dispute about the cell or cells involved in this early interval in the immune response. Earlier theories envisioned an arrangement in which antibodies were produced by cells that proliferated when they came directly into contact with foreign antigens. A general consensus now appears to be emerging that a phagocytic cell is also implicated in some way. This cell may have for its function the initial handling or processing of antigen. It may then pass on antigen or information about antigen to a cell identified morphologically as a small lymphocyte. Following stimulation through the intervention of a phagocytic cell, the lymphocyte undergoes differentiation and division with resulting production of antibody. The time during which antibody is rapidly increasing in concentration is called the productive or logarithmic phase. The amount of antibody produced rapidly increases to a plateau level and remains constant for a variable period of time, giving rise to the stationary phase of antibody formation.

*It remains possible, as Craddock[10] has stated, "that the products of lymphocyte transformation could assume the morphological and synthetic features of either plasma cells or lymphocytes containing cell-bound antibodies (i.e., able to mediate delayed hypersensitivity reactions) depending upon immunological circumstances." The suggestion that humoral and cellular immunity may be dependent on a common precursor cell has also been restated by Axelrad[11] and Schlossman.[12]

The humoral antibody response may be viewed as analogous to the neuronal reflex arc. It may be considered as consisting of afferent, central, and efferent component parts.[14, 14a] In the afferent component of the response there is interaction between antigen-capturing cells and cells with the potential for antibody formation. The central constituent is made up of immunocompetent cells, which are responsive to antigen. These cells are probably derived from the precursor cells with immune potential. The efferent part of the response involves the further differentiation and proliferation of immunologically competent cells with resultant antibody formation.

The above scheme provides an account of humoral antibody formation in which a functional description is given to participating cells. The cells may also be identified on the basis of the morphologic criteria of classical hematology (although this approach is at the risk of simplifying a complex sequence of cellular events).

Antigen, on entering tissue with antibody-forming potential, is initially encountered by mononuclear phagocytic cells. These cells of the reticuloendothelial system may be one of two types: (1) the circulating mononuclear phagocyte of the peripheral blood, the monocyte, or (2) the tissue macrophage found in locations such as lymph node, bone marrow, spleen, subcutaneous tissue, alveoli of the lung, adrenal gland, and serous cavities. The Kupffer cell of the liver is a cell of similar type. In the peripheral blood of man the cell representative of this series is the monocyte. Isolated and cultured in vitro on glass it can be seen to undergo differentiation to typical macrophage form.[15] A similar differentiation presumably occurs should the monocyte leave the circulation and settle in lymphatic tissue, on the surface of a serous cavity,[16] or in an inflammatory exudate.[17]

On the basis of animal experiments with isotopically labeled cells it is suggested that both the Kupffer cells of the liver and the alveolar macrophages of the lung derive origin from the blood monocyte. The monocyte develops by differentiation from a rapidly dividing pool of bone marrow cells called promonocytes. It enters the circulation from the bone marrow and passes from there in a random manner into extravascular tissue where it undergoes transformation to a macrophage form. In delayed-sensitive rats and guinea pigs the intravenous injection of the sensitizing antigen will cause an increase in blood monocytes (after an initial fall). The rise in monocytes results from the release of new cells from the bone marrow.[18] Cellular proliferation in the rat spleen increases after the uptake of a number of nonantigenic substances,[19] which suggests a mitotic event in the transformation of monocyte to macrophage. It is exceedingly unlikely that either monocytes or macrophages originate from lymphocytes.

The confusion and contradiction that center on this transformation may be because monocytes or promonocytes were mistakenly identified as lymphocyte-like cells. Human peripheral blood monocytes and hepatic and splenic macrophages carry an IgG receptor site on their cell surfaces.[20] This receptor is not present on lymphoid cells and may be a useful immunologic marker to distinguish mononuclear cells as being either monocytes or lymphocytes.

The eventual fate of macrophages once they are formed in extravascular tissue is uncertain. They may die in situ or possibly migrate back to the peripheral blood and recirculate. Some macrophages may persist in and about granulomata for periods up to 3 months without evidence of cell division, replacement, or death.[21]

The precise nature of the macrophage's intervention between antigen and antigen-sensitive cell remains to be defined. Experimental data to support the idea of some form of interplay between the two cells come from work with animals and observations of human cells in vitro. Groupings of lymphocytes about macrophages may be seen in the spleens of rats immunized with sheep red blood cells. Cinematographic techniques have shown peripolesis (movement over cell surface) and emperipolesis (entry into cell cytoplasm) of lymphocytes with regard to macrophages.[22] Lymphocytes may be seen to gather about macrophages and maintain prolonged contact with them through an extension of their cytoplasm called a uropod.[23] The uropod may actually penetrate into the cytoplasm of the macrophage. Thread-like structures or microspikes may extend from the uropod to objects with which the lymphocyte is in contact.[23a]

Electron microscopic examination of lymph node and spleen tissue taken from immunized rabbits has shown areas of cytoplasmic fusion between macrophages and lymphocytes.[24] A transfer of cytoplasmic contents was suggested. Phagocytic cells of the reticuloendothelial system may serve to capture and localize antigens within themselves by phagocytosis or pinocytosis or both. Following ingestion, the antigen may be degraded by enzymes associated with lysosomes.

Nossal has studied the uptake of ^{125}I-labeled *Salmonella* flagellar antigens by the rat lymph node. He has found antigen to enter macrophages that line the medullary sinuses of the lymph node by pinocytosis or phagocytosis. Antigen is then held in inclusions, some of which are lysosomes and some of which are more complex phagolysosomes. Antigenic determinants prepared by such processing might then pass to an antigen-sensitive cell in proximity and trigger antibody production. Nossal, however, suggests that these medullary phagocytes are scavengers or caretaker cells. He attaches significance to the localization of antigen in the cortex of the node on the membranes of macrophages with long dendritic processes. Such cells extend fine reticulum webs throughout the primary follicles of the lymph node cortex. The immunogenicity of antigen may be attributed to those molecules of antigen bound to the plasma membrane of macrophages.[26a]

The retention of antigen on the surface membranes of the cells would allow access to it by antigen-sensitive cells continuously moving in the neighborhood and so induce antibody formation. In fact, germinal centers do appear close to the dendritic macrophages that localize antigen on their surfaces. The ingestion of antigen or its retention on the membrane of a reticuloendothelial cell might be facilitated by antibody. Antibody may have opsonized the antigen before it reaches the lymph node or may be cytophilically attached to the phagocytic cell of the lymph node. It is possible that the phagocytic cell has specific surface receptors for immunoglobulin[26] and, therefore, picks up antigen coated with antibody. Macrophages do not possess an "immune memory." They may be more effective in

handling a bacterium on a second exposure to it, but this is because the first bacterium-macrophage contact induced the formation of cytoplasmic enzymes, which facilitate intracellular killing. The effect is nonspecific.

Other experimental evidence has accumulated to suggest that macrophages have more than a simple fix-and-hold function in the sequential cell action of phagocyte and antigen-sensitive cell that leads to antibody formation. It has been shown that extracts of ribonucleic acid (RNA) obtained from macrophages exposed to antigen in vitro will stimulate the in vitro production of antibody in lymph node cultures from nonimmune animals.[27] Subsequent work demonstrated that these preparations of RNA were not free of antigen. The amount of antibody was, however, still more than that which could be due to antigen alone.[28] The RNA might function as adjuvant, forming "super antigen."

On the other hand, the finding that the IgM antibody produced by the lymph node cells has allotypic markers peculiar to the macrophage donor supports the concept that the macrophage RNA is involved in the transfer of immunologic information and instruction.[29] The RNA to which antigen is complexed on entering the macrophage may be either a species of RNA already present in the macrophage or one that is newly formed in specific response to the antigen. The immunogenic function of macrophages has been shown to be radiosensitive,[30] and evidence has been presented to demonstrate that immunologic immaturity in the newborn mouse may be caused by a lack of macrophages rather than a lack of antibody-producing cells.[31]

The earlier concept that antibodies are produced by colonies of cells that divide when they come into contact with antigen has been modified in the light of the demonstrable interaction between antigen-sensitive cells and macrophages. The production of circulating antibody may be the result of an even more complex cell-cell interaction. Mitchell and co-workers[32] studied the 19S hemolysin response to sheep red blood cells in irradiated mice injected with a combination of syngeneic thymus cells and syngeneic bone marrow cells. A synergistic effect was obtained. The number of hemolysin-producing cells in the spleen of injected mice was greater when both types of cells were injected than when only one type of cell was injected. Under these conditions from 75 to 95% of the hemolysin-producing cells in the spleen were found to be derived not from the inoculum of thymus cells but from the bone marrow cells. The results raise the possibility that thymus cells and bone marrow cells function cooperatively. The thymus cells might contain a group of antigen-reactive cells that recognize and interact with antigen and subsequently stimulate the differentiation of hemolysin-forming precursor cells. Other antigenic systems require study before this scheme is accepted as a general phenomenon. In one such study thymus-marrow cooperation (similar to that found by Mitchell) was reported in the immune response of irradiated mice to bovine serum albumin.[33]

Other studies have shown that cells originating in the thymus will migrate to the spleen.[34] Such cells may divide in the spleen but are incapable of producing antibody.[35] Craddock[10] has combined these observations, together with the evi-

dence for macrophage-lymphocyte interaction, to formulate a hypothesis involving a three-cell system for induction of antibody production. He suggests a collaboration between thymus-derived cells (antigen-reactive cells?) and macrophages in the stimulation of antigen-sensitive cells to divide and produce antibody. Evidence for such three-cell interaction (antigen-sensitive cell, antibody-forming cell, and macrophage) was subsequently presented.[36]

Thymus-marrow synergism does not hold for every antigen. For example, in the irradiated mouse model, bone marrow without thymus cells will give an immune response to *S. adelaide* flagellin protein.[37] Therefore, antigens may be viewed as either thymus-independent or thymus-dependent, the two types of antigen being processed by different systems of cell interaction. Thymus-bone marrow cell interaction does not appear to be necessary for cell-mediated immune responses such as allograft rejection and graft-versus-host reactions.[37a]

While speculation and some uncertainty may still surround the early cellular events in the induction of antibody formation, a wealth of experimental evidence points clearly to the small lymphocyte as the immunologically responsive, antigen-sensitive cell or immunocyte—the cell ultimately responsible for antibody formation. This is not to say that the small lymphocytes constitute a homogeneous population of cells. For the sake of conceptual clarity, we have chosen to accept the distinction that has been drawn between thymus-dependent and immunoglobulin-producing populations of small lymphocytes. Morphologic criteria alone are not sufficient to distinguish between the two populations. If a separation is to be made, it must be on the basis of potential to undergo differing reactions of proliferation and differentiation in response to antigen. Our concern here is with the immunoglobulin-producing compartment of small lymphocytes.

The role of the plasma cell in antibody formation was established using tissue culture techniques.[38] This work was subsequently confirmed using single cell suspension studies[39] and immunofluorescence methods.[40] What remained unsettled was the question of the origin of the plasma cell. Specifically, did the plasma cell derive from the small lymphocyte, and could the lymphocyte produce antibody?

An examination of the morphology of single antibody-producing cells in the rabbit demonstrated that both lymphocytes and plasma cells formed antibody.[41] Antibody formation in rats was profoundly suppressed by prolonged cannulation of the thoracic duct and removal of lymphocytes.[42] Ability to make antibody was restored with transfusions of syngeneic thoracic duct cells. The above experiments established the humoral antibody-forming capacity of the small lymphocyte.

Two classes of cells were found with morphologic features identifying them as either lymphocytes or plasma cells through investigation of the electron microscopic morphology of 19S hemolysin-producing cells in the rabbit lymph node.[43] On the basis of size and development of endoplasmic reticulum, the cells observed could be arranged in series over a range from lymphocytes with the least developed such structures to plasma cells with the most mature form of this protein manufacturing apparatus. Lymph node cells obtained from the cisterna chyli of rabbits were mixed with antigen and placed intraperitoneally in diffusion cham-

bers. In the chambers plasmacytoid cells developed, which were thought to develop by "direct modulation of small lymphocytes."[44] In a series of cell transfer experiments lymphocytes were injected subcutaneously into rabbits, and by serial morphologic studies it was observed that the lymphocytes were replaced by mature plasma cells.[45] Little evidence of proliferative activity was noted at the transfer site, and it was concluded that the plasma cells developed from the transferred cells by differentiation. The development of cells with the morphologic appearance of plasma cells has been observed in cultures of rabbit lymphocytes stimulated with antigen.[46] These cells were intensely pyroninophilic and contained gamma globulin.

Experiments such as these, although not definitive, do provide strong inferential evidence for the view that the plasma cell is derived from a small lymphocyte. (The idea was probably originally advanced by Marschalko in 1895.[47]) Following appropriate antigenic stimulation, the small lymphocyte belonging to the immunoglobulin-producing compartment of lymphoid tissue undergoes transformation to a larger, more primitive cell. It is characterized by a fine chromatin pattern, nucleoli, and abundant basophilic cytoplasm. This cell, variously described as a lymphoblast, a plasmablast, a proplasmacyte, or a transition cell, is the immediate precursor of the plasma cell. The change from small lymphocyte to lymphoblast occurs by a process of differentiation. The transition from lymphoblast to plasma cell involves both further differentiation and proliferation. The plasma cell itself is an end stage cell and does not divide. The time required for the change from lymphocyte to plasma cell probably coincides with the logarithmic phase of antibody production.

The typical antibody response to a particulate antigen is marked by the production of IgM globulin (19S sedimentation) followed shortly thereafter by production of IgG globulin (7S sedimentation). Nossal[48] has studied antibody production by single cells following immunization. Individual cells were obtained by micromanipulation from the popliteal lymph nodes of rabbits immunized with *Salmonella adelaide* flagella. Early in the course of immunization, cells studied produced only 19S antibody. Cells forming 7S antibody were found at a later time. Cells producing both 19S and 7S antibody were noted at the time when a switchover from 19S to 7S antibody was observed in the serum. All cells examined were identified on the basis of morphology as being members of the plasma cell series. In this investigation, there was no relationship between cell immaturity and 19S antibody. Moreover, no morphologic distinction could be made between cells producing 19S or 7S antibody. It seems likely, however, that small lymphocytes and certain lymphoblasts produce 19S antibody and that the mature plasma cell produces only 7S antibody. The transition cell appears capable of manufacturing both types of immunoglobulin. There is evidence that the switchover from 19S to 7S antibody production occurs because of a feedback control mechanism, which is dependent on the production of a critical level of 7S antibody.[49]

Whether an antibody-producing cell is restricted to making a single antibody or whether it is able to respond with antibodies of more than one specificity remains controversial. It has been shown experimentally that some immunocompetent

cells are able to produce antibodies to at least two different antigens.[50] The small lymphocyte has the ability to respond immunologically to antigen either directly or after the antigen has been processed by a macrophage. It responds by first recognizing the antigen, probably by means of antibody, which it synthesizes and carries on its cell surface.[51, 52] The cell-surface antibody combines with specific antigen and triggers the further immunologic development of the small lymphocyte. Immunoglobulin determinants have been shown by mixed-antiglobulin reactions to be present on the surface of human lymphocytes.[52a] Human peripheral blood lymphocytes may carry on their surfaces determinant groups of the three major types of immunoglobulins.[52b]

The specificity of the antibody is governed by the genetic information contained in the lymphocyte's DNA. Genetic constitution in some species has been shown to determine the ability to respond to a particular antigen. A recognition step is probably necessary for the immunologic response of both thymus-dependent and thymus-independent lymphocytes. The cell-surface receptor for antigen need not necessarily be antibody. It may be some other, as yet undefined, molecule.[53]

In summary, the antigenically stimulated small lymphocyte with immunoglobulin-producing potential evolves through a series of differentiation and proliferative steps to a mature plasma cell. It passes through a phase in which, by conventional morphologic criteria, it has assumed a primitive blast cell appearance. During this stage in its metamorphosis, it will produce first IgM and then IgG antibody. At one point, it may manufacture both types of immunoglobulin. Once this cell matures to become a plasma cell, it is restricted to the formation of only IgG.*

Histology of humoral antibody formation

The principal functional units involved in the production of humoral antibody are the primary lymphoid follicles. These are defined by Miller and Nossal as being "rounded areas of densely packed small lymphocytes . . . which . . . contain no obvious primitive cells."[58] The penetration of antigen into these structures by way of afferent lymphatics appears to give rise to a secondary cellular formation—the germinal centers. These are described as "rounded collections of primitive lymphoid cells, macrophages, and reticular cells which develop following antigen deposition in primary follicles."[58]

*Some workers have offered evidence that cells producing IgM and IgG arise from separate precursor cells.[54, 55] In the lamina propria of the bowel, there is a population of lymphocytes and plasma cells which have been shown to produce and secrete largely IgA.[56] IgA is also the immunoglobulin most commonly found in lymphoid cells in the mucosa of the nose and bronchi, in the parotid gland, and in tonsillar tissue.[57] IgA is probably of importance in host resistance to microorganisms at body mucous membrane surfaces. IgM is important in the humoral immune response to gram-negative bacteria. Antibodies to gram-positive bacteria and to viruses are contained in the IgG fraction. The immunoglobulin IgE is made up of reaginic antibodies. The function of a fifth immunoglobulin, IgD, is not yet defined. Both IgE and IgD presumably take a cellular origin from lymphocytes (lymphoblasts, plasma cells). Generally, the relative frequency of a particular immunoglobulin-producing cell in lymphoid tissue is proportional to the concentration in the serum of the protein that it secretes.