Chapter 14: Basic Allergy and Immunology

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THUMB'S SECOND POSTULATE:

An easily-understood, workable falsehood is more useful than a complex, incomprehensible truth.

Arthur Block in MURPHY'S LAW BOOK II

The study of immunology is different from all of the other basic sciences because it necessarily involves an adversary system. The other basic sciences begin first with the homeostatic mechanisms of organs and tissues in their routine activities. They assume that an individual can acquire nutrients and the chemicals of life, transport them to the various parts of his body, and eliminate waste and toxic substances. By comparison, the study of immunology, like medical microbiology and infectious diseases, immediately implies that the individual is responding in some way to an encounter with another organism (or at least part of an organism).

Immunology in its broadest sense includes the study of infectious diseases, which has certainly been responsible for the major lifesaving advances over the last 100 years. The study of immunology could not proceed until the germ theory of disease had been established and now incorporates the most sophisticated studies of cellular biophysiology and biochemistry. Currently immunology includes many aspects of the study of cancer and the hemopoietic system. It is important that the otolaryngologist - head and neck surgeon has a sound understanding of immunology in a basic and clinical sense (Gluckman, 1982). This chapter deals with immunology both in a general sense and in its specific relationship to the area of the head and neck. Although a review of general principles of immunology is included, the reader is referred to general microbiology and immunology texts for a basic history and perspective of immunology, as well as details of many of its fundamental principles. To make this discussion most relevant to the student of head and neck diseases, it is written from a disease-oriented perspective rather than from the traditional chronologic review. This chapter centers on the disease states that highlight the importance of immunology and its role in health and disease.

Four basic disease states can be identified as the most dramatic immunologic failures. As illustrated in Fig. 14-1, these disease states define an "immunologic tetrahedron of life" within which the organism must maintain itself or succumb to an immunologic catastrophe. First, overwhelming infection is the most obvious immunologic threat. Sublethal infection has provided the basis for what is considered to be a normal and appropriate immunologic response. An inability to keep pace with infection, however, is a common cause of death even today. This apex of the tetrahedron is an example of a disease wherein the response is apparently appropriate. Cancer, on the other hand, provides an example of a disease wherein the response often is insufficient due to the ability of the organism to distinguish the "invader" from self is often lacking. The autoimmune diseases are an example of a state wherein there is a response with no apparent cause or preexisting disease. Allergy exists somewhere along the line between the appropriate response of the infectious disease state and

the overzealous response of autoimmune activity. Finally, the last apex of the tetrahedron is that of the severe combined immunologic deficiencies wherein there may be numerous appropriate stimuli, none of which are able to elicit an effective response.

Patients may succumb to overcolonization by infectious organisms or cancers cells. In a general sense, an organism must stay within the defined space of the tetrahedron to maintain life. It must respond appropriately to invasion from the outside by clearly defining the attacker as being foreign and assembling a response that can specifically neutralize the threat without injuring any of the normal physiologic processes necessary for day-to-day function. Transplantation and tumor chemotherapy are examples of iatrogenic effects that move an individual toward or away from the various apices of the tetrahedron. To help the reader find the more traditional topics of immunology discussed, various immunologic subjects are identified according to where they are discussed within the schema of the four disease states. Much of the normal physiologic response to an antigen is discussed in the section on infectious diseases; however, its significance is most dramatically identified when the response is absent, as in the immunodeficiency states. Thus this subject is also discussed in the section on the immunodeficiency syndrome. Current reports on cellular immunity and its role in oncology are cited to illustrate the discussion of the body's response to cancer. In addition, the role of biologic modifiers is discussed in the section on tumor immunology. The types of immunotherapy available are discussed under each disease entity as they pertain to that entity. The concept of ideotypes is found in the discussion of autoimmune diseases.

Infectious Diseases

General considerations

Vaccination of individuals to prevent common upper respiratory tract infections has probably contributed more to the general improvement of health in the last 100 years than any other medical discovery. A review of how diseases such as smallpox, diphtheria, pertussis, and polio affected the lives of many prominent individuals in recent history only emphasizes the importance of these diseases in the history of Western civilization.

Understanding how the cells process antigens to make antibodies was the key to understanding vaccination and lasting immunity. In addition, the discovery that antibodies can be used to identify specific molecules has rapidly advanced the investigation of many biologic phenomena. More recently, the ability to make monoclonal antibodies directed against specific substances has become the standard for many clinically significant diagnostic tests.

Definitions in immunology tend to be interdependent. For example, an *antibody* is a protein that is produced as a result of exposure to an antigen and that has the ability to combine with this same agent. An *antigen,* on the other hand, is a substance that can induce a specific, detectable, immunologic response when introduced into an animal; and the experience is chemically remembered, at least for some significant time.

Because these definitions are interdependent, one must stand outside of the system just slightly to observe some other phenomena that have been known for a long time. Fortunately for early investigators, antibody molecules are relatively plentiful in the sera of mammals and fowl. Early attempts to separate serum proteins into different groups by electrophoresis

identified the fourth globulin fraction from albumin as the one that contained molecules that could combine with specific antigens. So named for its electrophoretic positions (there were two alpha peaks), further study identified subclasses of "gamma-globulins" and led to the identification of the immunoglobulin molecules. There are five major classifications of immunoglobulin molecules: IgM, IgG, IgA, IgD, and IgE. Although little is known about the IgD role in immunologic responses, each of the other subgroups has more or less specific types of reactions or areas in the body in which it appears to be most beneficial.

The original conceptualization and descriptive terms of the molecule came from experiments using chemical agents that disrupted disulfide bonds, demonstrating that the basic immunoglobulin molecule (that is, the IgG molecules) was made of four chains. Stoichiometric analysis determined that these four chains appears as two pairs: one large, or "heavy", with regard to its sedimentation coefficient, and the other small, or "light". The type of heavy chain determines the basic type of immunoglobulin molecule, and two types (kappa or lambda) of light chains appear with a variety of heavy chains. Each plasma cell that makes immunoglobulin makes basically only one type of light chain and one type of heavy chain, but its heavy chains may change over time. Natural immunologic stimulation produces a proliferation of cells that have both kappa and lambda chain production in the population of responding cells. An abnormal proliferation of antibody-producing cells arising from one malignant transformed precursor cell (as occurs in certain lymphomas) results in only one type of light chain expressed on all cell surfaces. The detection of surface-bound antibody is performed using an anti-antibody made by injecting another species with purified human antibodies as diagrammed in Fig. 14-2.

The light and heavy chains are held together with disulfide bonds, so that a single heavy chain and a single light chain make an Ag recognition site. The heavy chains are attached to each other, so that the molecule has symmetry if divided down its long axis. In Fig. 14-3, one can appreciate the general diagrammed structure of an immunoglobulin molecule, which has an antibody-combining end (Fab) and an opposite end (Fc) that can combine with a cell surface receptor. Digestion with proteolytic enzyme (papain) yields a preparation that divides the immunoglobulin molecule by cutting the heavy chains between the interchain disulfide bonds that connects the two heavy chains. This gives one preparation that can combine with antigens and another that may bind to the surface of cells. It was determined that this opposite end could crystalize under the proper conditions. Hence it was termed the *Fc fraction,* and the receptor to this end of the molecule were referred to as *Fc receptors.* Fc receptors are found on many cells of the immune system that are drawn to an area of inflammation by chemically recognizing the Fc end of an antigen-antibody complex.

The antibody-combining end is technically referred to as the $F(AB^1)_2$ segment, or more loosely, the *Fab* end of the molecule. Under special reducing conditions, the $F(AB^1)_2$ can be divided into two identical fragments, each of which can combine with a single antigen. After the identification of this antibody structure, many of the properties of the immunoglobulin molecules became clear.

The first step was to determine the number of antigen-combining sites per molecule that exist for IgG, IgD, IgE, and most of the serum components of IgA. We now know they contain the basic four-chain immunoglobulin subunit shown in Fig. 14-3 referred to as a *monomer.* Both IgA and IgM tend to form larger complexes of these basic subunits, the

secretory IgA being primarily a dimer and serum IgM being a pentamer; but for general serum immunologic evaluations, it is useful to consider the antibody molecules as having two antigen-combining sites on a single molecule (that it, a monomer with a valence of two).

When large particles, such as blood cells or bacteria, are used as the antigen and mixed with antibody molecules, the antigen and the antibody can combine in one of three relative patterns. If one considers a constant amount of antigen, one can have low, optimal, or high concentrations of antibody relative to that specific antigen. It is only with optimal concentrations of both antigen and antibody that agglutination occurs and (in situations where soluble antigens are used) precipitation can be identified.

For simplicity, this situation can be imagined to happen when roughly equal amounts of antigen determinants and antibody exist. In the circumstance wherein relatively low amounts of antibody exist in comparison with the antigen, an antibody molecule that does combine with the antigen may allow a small number of antigen molecules to be connected, but not enough are present to form an obvious latticework. If this type of stoichiometry occurs in plasma, the antibody-Ag complex circulates until filtered by the kidney where an inappropriate immunologic response is directed. Similarly, when a great excess of antibody exists, the antigenic sites on a large object are all occupied by antibody sites; however, when adjacent antigens come near enough to allow the other arm of the antibody to combine with the antigen, it finds that the sites on those other antigens are already occupied by antibody molecules. Thus neither lattice formation nor precipitation will occur.

In the midrange, where there are optimal concentrations, it is easy to imagine how an antibody can bridge two antigens, holding them together and allowing both lattice formation and precipitation to occur. Under this circumstance, virus and bacteria are immobilized where they can be attached to phagocytic leukocytes. In such a plate, agar is used as the medium that proteins can diffuse. Two or more wells are cut in the film of agar; antigen is placed in one well and antibody in the other. As the molecules diffuse through the agar according to their size and solubility, molecules begin to meet in the field between the two wells. Where antigen and antibody concentrations are optimal, precipitation occurs. The reaction can be made considerably more complex by including additional antigens or antibodies to test the specificities of the chemicals at hard or by separating complex antigens with electrophoresis or chromatography techniques. After the wells are washed and non-precipitated protein eluted from the gel, the gels can be stained for protein-identifying presence or absence of antigen-antibody precipitate, which identifies specific precipitated antibody as a stable complex.

This concept of antigen-antibody concentrations observed in gels also has a direct clinical counterpart, as suboptimal concentrations of antigens and antibodies can lead to nonprecipitating circulating antigen-antibody complexes that may injure the kidneys and other tissues or immunosuppress the host. For discussions in greater detail on the production of antibodies, the nature of their combining specificity, and assays, the reader should refer to texts and journal articles on the subject of circulating immune complexes (Schantz, 1988).

IgA

Because IgA is secreted by many tissues of the head and neck, it is included in this discussion. IgA is certainly the principal class of immunoglobulin in most external secretions

(Lamm, 1976). It is made by plasma cells in glands and mucous membranes that oppose the outside world. IgA occurs in primarily two forms: a serum form and a secretory form (known as *secretory* IgA). Secretory IgA has a distinctive extra polypeptide chain (known as the *secretory component*), which helps it pass through cell membrane barriers. In addition, the two monomers of the IgA molecules that make up the secretory dimer are held together by a J chain. This J chain also exists on IgM molecules (Fig. 14-3). J chains appear to be important in the assembly of polymeric forms of the immunoglobulins. The J chain is covalently bound to monomers and allows then to acquire new biologic properties. Although the J chain is bound to the Fc portion of the molecule, it does not appear to be important in complement-fixing activity.

J chains are synthesized in the same plasma cells that secrete polymeric immunoglobulins (Koshland, 1975). Both intracellular and extracellular monomeric IgA lack the J chain. It appears that only cells that are involved in secreting the dimeric form of IgA use J chains. Monomeric IgA-secreting plasmacytomas do not secrete J chains. This situation emphasizes that the Fc portion of the IgA antibody appears slightly different from that of the other immunoglobulins.

It is important to note that IgA is a first-line defense system that protects the body against invasion by microorganisms and the entrance of foreign molecules. The monomeric form of IgA does not make a significant contribution to IgA in secretions (Lamm, 1976) (Fig. 14-4). Similarly, IgA antibodies in secretions are not absorbed into the circulation. This fact is important in evaluating the value of IgA antibodies in human mother's milk. Because they are not absorbed into the bloodstream from the digestive tract, IgA antibodies have to exert all the beneficial effect locally in the oral cavity, pharynx, or lower digestive tract. IgA molecules do not mediate anaphylaxis, chemotaxis, or cytophilia. In this way, IgA antibody can be contrasted to IgE, where these reactions seemed most marked.

Another important concept about IgA is that the constant region of the alpha-chain (the Fc portion) cannot fix complement. The significance of this concept is examined again in the discussion of the immunology of cancer. IgA antibodies can activate the alternate complement pathway if aggregated; however, this does not appear to be a physiologic phenomenon. The ability of IgA antibodies to stimulate opsonization is controversial but certainly much less than that of IgG. IgA's main activity is as a protective effect against microorganisms and foreign substances in the digestive tract. This is borne out by observation that IgA-deficient persons have both quantitative and qualitative increases in humoral antibodies directed toward antigens associated with food and intestinal organisms. Secretory IgA antibodies are quite stable and resistant to digestion. They, like all other antibodies, are not permanently denatured by pH2 of the stomach and pass through the digestive tract relatively unchanged, thus retaining their activity.

IgA can usually be found in external secretions, such as saliva and tears. Normally the levels of IgA in the serum of infants are low and are thought to be synthesized by the fetus, as IgA does not appear to cross the placenta. The IgA system is slow to mature in the infant for reasons that are not well understood. Evidence suggests that cells switch from secreting IgM to IgA, as with the other immunoglobulins; but investigation in this area has not been completed. Secretion of IgA does seem to be a terminal event in cell differentiation; however, whether intermediate steps occur is unclear. Secretions of IgA by various mucous membranes

and glands appear to come under local control. In tissues that do not secrete IgA, an abundance of plasma cells secreting IgA can be identified and predominate markedly over those secreting the other immunoglobulins. Adenoids are large producers of secretory IgA (Lamm, 1976).

In germ-free animals that are immunized parenterally, the major plasma cell response is to make IgM antibody and, after several immunizations, IgG. In contrast, when these animals are immunized by the oral route, the major plasma cell response is to make secretory IgA locally and serum IgA.

After oral Sabin polio vaccine administration, the nasopharynx and intestine can be observed to excrete IgA without a significant IgG or IgM antibody response. In contrast to the observation of serum antibodies after parenteral (Salk) vaccination and when the respiratory tract is directly immunized against influenza virus, no secretory IgA antibody against influenza virus can be found in saliva.

Thus it appears that once small lymphocytes have differentiated, the plasma cells are committed to secrete IgA antibody. They tend to remain in the mucous membranes where the antigen was encountered and generally do not migrate to the other areas of the body. The quantitative difference between specific secretory antibody as opposed to that which appears in serum has raised questions about local synthesis. Specific secretion of IgA antibody sometimes can be disseminated to distant mucous membranes. For instance, antipneumococcal antibodies can be seen in human colostrum. It is presumed that some IgA-producing B cells must migrate from one mucosa-associated lymphoid tissue to the breast tissue for this phenomenon to occur.

The mechanism by which the IgA antibody molecules crosses the basement membrane for secretion is partially understood (Stites et al, 1984). The most likely anatomic route for IgA to exit the body is for local plasma cells to secrete into the intraepithelial space, then for IgA to migrate to the interior of the epithelial cell and finally to pass into the lumen of the secretory organ. The secretory component appears to be synthesized by the epithelial cells that line the mucous membrane, ducts, and glandular acini of the body. Other work also supports the concept that the secretion of IgA is a combination of two distinct types of cells. The secretory component is found only in the columnar cells lining the mucous membranes and in the serous acini cells of glands. A variety of mechanisms by which the final assembly of secretory IgA actually takes place have been proposed. The actual site may include the intraepithelial space, the plasma membrane of the epithelial cell, and the interior of the epithelial cell itself. Movement through all the areas appears to be active, as passive absorption of secretory IgA back into the serum of newborns receiving IgA in colostrum is not observed.

Once IgA is inside the epithelial cell, secretion must occur by processes that are similar to the secretion of other proteins. Vesicles are formed that originate from the rough endoplasmic reticulum and subsequently move through the Golgi apparatus. Granules that contain secreted material then arrive at the surface, where they are discharged.

The mechanism of IgA secretion is shown in Fig. 14-4, which also shows how other antibodies get into the pharynx during an acute infection. Epithelial cells separate during

inflammation and allow a leak of IgM and IgG. The diagram also demonstrates that adenoids and tonsils are composed of different types of surface cell. Adenoids have ciliated respiratory epithelial cells that can secrete IgA; the epithelial cells of tonsils (along with the rest of the lower pharynx) cannot. The tonsil processes antigens and sends plasma cells to other antibody-producing sites through vascular antigen recognition.

The local plasma cells that secrete dimeric IgA must bear an intimate relationship with the epithelial cells. IgA production is under the same T-cell control system as that of the other immunoglobulins (see Control of Antibody Synthesis). Circulating IgA appears to be particularly dependent on thymus maturation, as circulating IgA is deficient in humans with thymic dysfunction. These individuals also often exhibit diminished secretory IgA. Decreased production of IgA associated with thymic aplasia is included in the discussion of the acquired immunodeficiency syndrome (AIDS). Overproduction of IgA is uncommonly seen but may be observed in certain infections that give rise to an enormous mucous membrane inflammation, such as cholera.

Control of antibody synthesis

Cells that produce antibody arise from multipotent stem cells, first in the hemopoietic tissue of the liver of the fetus and then in the bone marrow as hemopoiesis shifts from one site to the other later in development. Surface antigens of the class II MHC series and other antigens identify lymphocytes of the B-cell lineage. The first differentiation is to a pre-B cell, which attains the ability to produce IgM as differentiation proceeds. Because there are several hundred variable regions on heavy chains and somewhat less than a tenth of those in the diverse region of light chains, the various combinations can produce a multitude of different protein sequences when these chains are switched and rearranged (Schwartz, 1984). In an initial differentiation of a pre-B cell, one set of these chains is selected and aligned for transcription. Actually, these immature cells produce no immunoglobulin, but once they differentiate, M chains (that is, the heavy chain for IgM) can be observed in their cytoplasm. A similar process occurs for light-chain rearrangement.

Once this differentiation step is initiated, an immature B cell with membrane-bound IgM can be identified. Surface IgD can also be observed in many of these cells. After this basic synthesis takes place, nuclear arrangement of the DNA of the so-called constant portion of a molecule can be switched genetically by splicing one region that codes for an IgM heavy chain with another heavy chain portion, so that an antibody molecule with the same relative antigen specificity can be switched from IgM to IgG, IgA, or IgE, or to any of the subtypes of IgG or IgA. Generally, it is thought that lymphocytes produce only one type of immunoglobulin, but during the switchover, B lymphocytes can express as many as three different immunoglobulin isotypes, simultaneously. After these cells are formed, they migrate to the circulation and to areas of antibody synthesis.

Differentiated B cells live for only a short time and need to be regenerated if that clone of cells is to persist and significant antibody production is to continue. The class II MHC surface components of these cells help T cells and others recognize them and are important for activation of the resting B cells. For B-cell activation to occur, the appropriate antigen combines with surface antibody on the B cell and with a similar receptor on helper T cells. The T cell antigen can then interact with the B cells by producing soluble signals (IL-

6) for proliferation and cell differentiation into plasma cells. (This phenomenon is discussed again in the section on tumor immunology.)

Not all activated B cells go on to produce terminally differentiated plasma cells, but some serve as relatively long-lived memory cells. These memory cells are relatively easily triggered by antigen and are responsible for the anamnestic response seen after the second exposure to the antigen and for the clinical phenomenon of lasting immunity. When the B cell finally differentiates into a plasma cell, it loses its class I MHC antigen and surface immunoglobulin as it begins to make genetically directed antibody. These cells divide only rarely and live for only a limited time under normal circumstances.

The general process of the ability of B cells to produce one or another antibody also matures with age. As mentioned previously, the IgA antibody response is the last to mature. Immature B cells express surface IgM molecules and other membrane glycoproteins, including the receptor for complement component C3b. They are easily inhibited by surface IgM cross-linkage when they are immature, but the same stimulus applied to a more mature cell induces the cells to proliferate. The mechanism by which this feature helps regulate the growth potential of these cells is discussed in the section on autoimmunity, which includes the concept of idiotypes.

Complement system

It is important to discuss the complement system in combination with antigen-antibody reactions because it is the primary system by which antigen-antibody reactions can be cytolytic. In addition, components of the complement system can recruit a cellular inflammatory response by a variety of mechanisms. For detailed explanations of the complement system, the reader is referred to texts on this subject (Parker, 1980; Stites et al, 1991). A brief review is included here of the two similar but interdependent pathways leading to activation of the complement system (Fig. 14-5).

The two pathways are triggered by different chemical mediators. The first, referred to as the *classical complement pathway,* is activated by antigen-antibody complexes or aggregated immunoglobulins. Immunoglobulins IgA, IgD, IgE, and some special subclasses of IgG are inactive with regard to the ability to activate complement. To begin the process, the Fc region of an IgG or IgM molecules finds the first component of the complement system (C1) and initiates the following cascade of events. (The pathway can also be activated by other chemical mediators, including DNA, C-reactive proteins, proteolytic enzymes, and some cellular membrane components.) After initiation, the pathway is activated by a series of enzyme substrate reactions and protein interactions, resulting in the formation of complement enzyme complexes. The reactions diagrammed in Fig. 14-5 are quite specific, and molecules that can activate the system between C1 and C3 have been identified. The reaction products of the system are in a sense, a "footprint" of activation of the immune system and can be assayed to detect immune activity in patients.

The alternate pathway, referred to historically as the *properdin pathway,* can be activated by lipopolysaccharides, cobra venom factor, and proteolytic enzymes, as well as by IgG and IgA. The properdin system was discovered independently of the classic complement system and for a while was thought to be separate from it. It was named properdin because

it allowed the individual to be "prepared" for destruction and neutralization of certain bacteria and viruses without previous immunologic experience. Obviously, antibody is not necessary to activate this system; this is the mechanism by which patients with paroxysmal nocturnal hemoglobinuria lyse erythrocytes. The pathway is initiated by another component of the classic system, C3b, which is thought to be released in small amounts in the body at all times. Other serum proteins usually rapidly inactivate C3b and maintain a steady-state, low concentration of this molecule. In some circumstances, however, C3b production is increased and bound to cell membranes, which protect it from serum-neutralizing proteins. This product can in turn bind with factor B, making a C3b, Bb surface enzyme complex, which, when reacted with factor D, is able to generale large amount of C3, and is in that sense a self-amplifying system. These activated proteins bind to membranes, converting CS to membrane-bound CSb. From there on, both pathways use the same reaction. The continued cascade of enzyme substrate reactions and proteins interactions leads to membrane leakage and eventual osmotic destruction.

A number of serum proteins modulate or limit the activation of the complement system to keep it confined to localized activity. Among these is C1 esterase inhibitor, which inhibits both C1 and the initial steps in the kinin-forming and blood-clotting pathways. Factor 1 inactivates C3b in solution, and anaphylotoxin inactivator destroys the biologic activities of C3a-, C4g- and CSa-cleaved fragments of C3, C4, and CS. The S protein binds to the Csb,6,7 complex, modulating the activity of final sequence, which is membrane bound. Many of the complement system proteins are heat inactivated, and this method is commonly used to treat serum when noncomplement activity is to be assayed. Complement activity in serum can be measured by testing the ability of a given dilution of serum to lysed sheep red blood cells treated with complement-fixing anti-sheep antibody. This is usually expressed as a 50% hemolytic complement unit per milliliter, which is the reciprocal of the dilution (amount) required to lyse 50% of the test cells. Similar systems can be used to detect complement system components or inactivators of this system.

There are several biologically significant ramifications of the complement system in addition to that mentioned previously. First, certain low molecular fragments of C3, C4, and CS (C3A, C4A, and CSA) act as anaphylotoxins, inducing smooth muscle contraction and a vascular permeability and histamine release from mast cells and basophils. The extent to which the complement system can exert its biologic significance is demonstrated in experimental models when complement-fixing antibody to tissue, such as glomerular basement membrane, is injected into an animal. The local damage to the kidney, which includes cell lysis, polymorphonuclear leukocyte infiltration, and basal cell degranulation, can be attributed to the complement system. Glomerulonephritis and autoimmune diseases, such as rheumatoid arthritis, are common examples in humans.

Loss of inactivators of the complement system can be equally dramatic in showing the power of the complement system. Inflammatory angioedema, which involves congenital deficiency of C1 inactivator, leads to uncontrolled activation of the classic pathway in the clinical syndrome of hereditary angioneurotic edema. Deficiencies of the classic pathway have also been identified. Affected individuals suffer from a variety of diseases, including systemic lupus erythematosus, glomerulonephritis, and repeated infections.

Allergy and Hypersensitivity

Perspective

The clinical study of allergy has been separated from classic immunology by its language, but the basic science observations, which allowed elucidation of many basic science phenomena, owe their observations to early-observed immunologic phenomena (Johansson and Bennish, 1982). Even the original observation of passive transfer of immunoreactivity with serum, the Prausnitz-Kustner (PK) reaction, was performed with serum from a patient with fish allergy and is a fundamental immunologic phenomenon. This discussion places allergy or hypersensitivity on the tetrahedronm between infectious diseases and autoimmune diseases. Indeed, several autoimmune diseases are classified as hypersensitivity-type phenomena. An attempt has been made to organize immunologic diseases and name them with Roman numerals, which helps organize them into clinical entities very little and helps the student of allergic diseases not at all. This chapter deals with phenomena as they are observed clinically, using descriptive nomenclature wherever possible. For those who enjoy making simple communication complex, the following classification translates one descriptive term into the nondescriptive arbitrary classification.

Classification of immune mechanisms of tissue injury

Type I	Anaphylactic - IgE mediated
Type II	Antitissue (literally "antibody" - directed antibody, which is cytotoxic)
Type III	Immune-complex deposition - complement mediated
Type IV	Delayed hypersensitivity - T-cell mediated.

The numerical classification may become rapidly outdated (in terms of understanding how these processes come about), as it is likely that the underlying pathophysiology for many of these diseases lies in activities of cell populations that are yet to be clearly identified and characterized. When such identification occurs, a more appropriate name that conveys an understanding of the disease process will be applied.

The early study of allergic diseases brought into use many terms that are no longer applicable. The concept of reagin and reaginic activity has been replaced with the concept of IgE serum protein, which mediates this activity. Similarly, antigens that give rise to allergic phenomena are often referred to as "allergens". Since allergens are clearly antigens that are not fundamentally different from those that induce other immunoglobulin responses, this discussion refers to these foreign substances as antigens, and physicians should interpret them as those that give rise to allergic immunologic phenomena. This discussion tries to unify concepts of immunology rather than create the impression that multiple unrelated phenomena occur. To this end, this section discusses components of allergy in the terms of the basic immunologist.

For the most part, a majority of diseases thought of as "allergic" can be split into two groups: those that are IgE (indirectly beta-cell) mediated (type I reactions) and those that are T-cell mediated (type II reactions). Two other major immunologic disease classifications must also be mentioned. Diseases that have a primary antibody and cell-mediated immunity

directed toward one's own tissues (such as seen in the connective tissue diseases) are seen with some regularity in the head and neck area because they involve primarily or secondarily the secretory glands of the head and neck, the temporomandibular joint, the thyroid gland, or general vasculature. This is probably the truest use of the term *antibody,* as the immunoglobulins react with one's own body in type II reactions. Examples of these diseases in the head and neck are listed in the discussion on autoimmune diseases. Immune complex diseases mediated by complement that involves the deposition of antigen-antibody complexes in tissues (type III reactions) have only a few applications to the physician. Only IgE production is discussed here. Delayed hypersensitivity can be interpreted as cell-mediated immunity, which is included in the discussion on tumor immunology.

IgE production

It is not clear why some individuals seem to form clinically significant amounts of IgE antibody, whereas others do not. Nor is it clear why some antigens appear to elicit more IgE response than others. It has been clearly established that for IgE production, both T and B lymphocytes are involved in the control of IgE formation (Buckley et al, 1982). One subset of B lymphocytes actually synthesizes IgE; however, the same type of T cell-dependent clonal perforation and B-cell differentiation is necessary, as it is for all of the other antibody classes.

The catabolic rate of serum IgE is very short, with an average half-life in the serum of 2 to 3 days. Recalling that the amount of time required for loss of maternal IgG protective effect in newborn is measured in months, it is obvious that the half-life of IgG is much longer. Antibody bound to mast cells may persist longer than unbound IgE. Such observations do suggest that IgE antibody is being formed continuously, and the presence of the antigen or sensitizing molecule is necessary to keep levels at clinically significant titers. This has been shown for ragweed antigen, where the IgE antibody titers follow exposure to the antigen and are increased during and after the ragweed season and then fall off somewhat over a period of months (Mathews, 1982). Secondary anamnestic responses can be seen with IgE production that are similar to those observed with IgG. This is in part responsible for the phenomenon of individuals being able to tolerate a foreign molecule at one time and not another. The dramatic clinical stories associated with penicillin allergies are common examples.

IgE-forming cells were first detected in primate tonsils and adenoids that had been removed because of recurrent infections. Tonsils and adenoids are stores for large numbers of plasma cells; therefore, a good proportion of the germinal centers leukocytes may stain with anti-IgE antibody. In contrast, the subcutaneous lymph nodes and spleen are observed to have only a few IgE-forming cells. IgE-forming cells have also been identified in the nasal mucosa and bronchial mucosa, especially in and around the minor salivary glands. They have also been observed around the intestine; however, lymphoid cells and bone marrow, lung, and peripheral blood cells do not stain with anti-IgE.

Many agents do produce an IgE response, but the degree of response varies among them. For instance, the IgE antibody response to ragweed antigen is well known, but individuals with hay fever often also have IgE antibody to a number of other antigens. This leads to the concept of common "IgE-selecting" immunologic properties of antigens, although none has been identified other than the fact that they have high immunogenicity (Mathews, 1982).

Helminth infections, particularly with extract of *Ascaris lumbricoides,* have long been shown to be a significant inducer of IgE-type reaction. This fact probably in part accounts for the increased eosinophil count in patients with parasitic burdens. The dose of the antigen also seems to be important. Experiments with rats have shown that small to intermediate doses are more successful at producing IgE antibody response than are high doses. This fact seems to be particularly important when hapten is used.

The possibility of IgE-binding factors potentiating or supporting IgE production has been investigated (Ishizala, 1982a). These factors have a high affinity for IgE electively and enhance a differentiation from IgE-B cells to IgE-forming cells. Experiments have shown that smaller amounts of T-cell mitogens are IgE-binding factor suppressive as opposed to higher amounts, which are IgE potentiating. The relationship between the amount of T-cell mitogen and antigen appears to be reciprocal, but in vivo analysis of this relationship has not been studied. Nevertheless, it is useful to think of both humoral and cellular responses that can control IgE synthesis to obtain a desirable homeostatic control.

Another line of evidence for T-cell regulation of IgE production comes from observation of patients with partial thymus-dependent immune deficiencies. These diseases, which are discussed in detail in the section on immune deficiency, often demonstrate increased IgE production with high frequency in many of the patients (Buckley et al, 1982). This is postulated to occur because of a deficiency of T cells that regulate (suppress) the IgE responses.

The thymus-dependent lymphocytes, which are responsible for IgE antibody production helper function in mice, have a special surface antigen and can be distinguished from other helper T cells that support IgG or IgM production. IgE response involves B cells with memory in the same manner as other antibody responses and are distinct from those for IgM or IgG antibody. There appear to be different soluble enhancing factors for IgE and IgG antibody, at least as observed in mice. Prevaccination injection of an animal with IgG or IgM antibody to a particular antigen will suppress IgE formation after vaccination, as it does for IgG.

IgE-mediated response

A variety of chemical mediators are released when the mast cell degranulates. Among them are histamine, leukotrienes (formerly, "slow-reacting substance of anaphylaxis (SRS-A)"), eosinophilic chemotactic factor of anaphylaxis, heparin, superoxide dismutase, prostaglandins and thromboxanes, platelet-activating factor, neutrophil chemotactic factor of anaphylaxis, bradykinin, and a number of enzymes, such as chymotrypsin, trypsin carboxypeptidase A, glycosidases, and sulfatases (Ishizaka, 1982a, 1982b). All of these are thought to have some immunologic activity.

It is probably useful to imagine that any mast cell degranulation causes a local effect that varies depending on the area in which the cells are located. Since different tissues have different responses, one can identify that histamine, leukotrienes, acetylcholine, and bradykinin are partially responsible for bronchospasm; histamine, leukotrienes, and prostaglandin E are responsible for mucosal edema; histamine and eosinophilic chemotactic factor are responsible for eosinophil infiltration (Fadel, 1990); neutrophil chemotactic factor

of anaphylaxis and other inflammatory factors derived from mast cell granules are responsible for neutrophil infiltration; histamine, acetylcholine, prostaglandins, and alpha-adrenergic agonists are responsible for mucous secretion; and the hydrogen peroxide radicals and proteolytic enzymes are responsible for desquamation and basal membrane thickening.

The complex nasal response, which has been investigated and reviewed by Naclerio et al (1985), involves histamine, TAME-esterase activity, kinin, prostaglandin P2, and leukotriene C. Intravenous antigen exposure may allow all of these to occur, causing anaphylaxis (Patterson and Valentine, 1982). IgE has the same relative specificity for antigens as do the other immunoglobulins. Its only real difference is its ability to cause mast cell degranulation. The initial histamine response is only part of the allergic reaction to an allergen. A late response not blocked by antihistamine causes nasal congestion.

The production of IgE antibodies and the formation of the active sites involved in these molecules are quite similar to that already discussed for IgG. Many experiments have been performed using the $F(Ab^1)_2$ and Fc portions of the molecule. The way the IgE molecules react indirectly with each other and with mast cells differentiates IgE responses from the other classes of antibody and antigen-antibody reactions with them. Antigen-antibody complexes that involve two antigen molecules and one IgE antibody molecule do not induce typical IgE reactions. However, complexes that allow two IgE antibody molecules to react with the same antigen molecule can induce such reactions.

Current theories of mechanisms of cell-bound IgE and IgE receptors involve the phenomenon of two adjacent IgE molecules sharing attachment on a common antigen, which directly activates membrane-associated enzymes. This phenomenon has been studied indirectly by making antibody molecules to the IgE receptors (Stites et al, 1982). Because it is the F(AB) portion of the molecule that will combine with the receptor (and not the Fc fragment), the antibody that is made to the receptors can be of any class. It is not limited to IgE. Calcium influx, which is a measurement of the mast cell degranulation phenomenon, in addition to histamine release, is observed when the intact antireceptor antibody used is made up of the divalent portion of the molecule, $F(AB^1)_2$, but not when the monovalent form of the molecule F(AB) is used. This indicates that two adjacent IgE molecules must react with an antigen to cause mast cell degranulation.

Because antireceptor antibodies are not a natural occurrence, it is the heavy-chain specificity of IgE bound to IgE receptors that determines physiologic response. The triggering of histamine release through IgE receptors is mediated only by IgE antibodies. The calcium influx mentioned earlier is seen as an essential initial step in mast cell activation before histamine release. Activation of methyltransferase, which stimulates phospholipid methylation in the plasma membrane, follows bridging of IgE receptors on mast cells. This response occurs within about 15 seconds after antigen-antibody combination. Similarly, an intracellular cyclic AMP (CAMP), rising in approximately the same time sequence as phospholipid methylation, indicates a close relationship between IgE receptors and both adenylate cyclase and methyltransferases. It also explains why theophylline can directly inhibit skin testing, as AMP is involved in the primary stage as mast cell degranulation.

Allergy testing

A variety of methods are used to assess an individual's allergic status. They are divided here into in vivo and in vitro tests.

In vivo testing

The most obvious in vivo test is to expose the organism (patient) to a stimulus that is thought to be the antigen (allergen) and observe the reaction, whether it be bronchospasm or rhinorrhea. Because there are a large variety of potential antigens and because one cannot test sequentially once a generalized reaction has been initiated, such tests have limited clinical applicability. Antigenic challenge is useful in confirming other test results that may be equivocal before therapy is started. A diet that removes all potential antigens and returns them sequentially may be useful in the evaluation of food allergy (Anderson, 1984; Buckley and Mathews, 1982). The patient's safety must be carefully considered in recommending an antigenic challenge.

Skin testing is a form of in vivo testing that uses a relatively safe response organ for assessment. (This testing assumes that the response is confined to the skin and does not become generalized or systemic.) It is important to run proper controls during this testing to rule out nonspecific reactions. The tests can be performed in two ways: pricking the skin or interdermal injection. With the skin prick test, the skin is gently pricked underneath a drop of antigen solution (1:10 weight/volume), and any reaction should develop within approximately 20 minutes. Local edema and erythema (wheal-and-flare reaction) are positive results. The main advantage of this method is the safety involved, as the reaction can be observed with an extremely small amount of antigen, and the possibility of a systemic reaction occurring during testing is small.

With the interdermal test, usually 0.02 Ml is injected into the superficial layers of the skin. The test solution is usually two to three orders of magnitude less concentrated than that for the prick test. With the interdermal test, the quantitation of the size of the reaction can be made more accurately, as a known amount of antigen is administered, in contrast to the skin prick test. A skin test that essentially injects small quantities of antigen into the skin has been standardized by determining the concentration of the antigen present and correlating this amount with the degree of reaction that occurs. This approach is the basis of the Rinkle technique, from which a safe starting dose of antigen can be estimated. The serial dilution technique may provide improved therapeutic results from some individuals (Draper, 1979). Obviously, medicines that interfere with allergic reactions will modify the results. The adrenergic agonist, aminophylline, and antihistamines interfere with such test (Simons, 1990). Interestingly, steroids do not seem to interfere with this evaluation.

Because many patients are not sure about the exact antigens involved, often a battery of testing must be performed. This fact, complicated by the concern over systemic reaction in the interdermal test, make such testing complex, requiring a judicious choice of antigens and experienced personnel. Still, skin testing has remained faster, less expensive, and probably more sensitive than the following methods, which use serum analysis.

In vivo tests that qualitatively assess serum IgE are as old as the concept of IgE. IgE can be identified by passive transfer of allergic phenomena using serum from a patient injected subcutaneously into a second individual followed by antigen challenge. This reaction (the PK reaction) requires specific controls in a matched cooperative test individual and is not practical in most clinical circumstances. Nevertheless, it provides insight into the mechanism of allergic phenomena and is a close corollary of Koch's postulates for infectious diseases.

In vitro testing

Recent interest has centered on evaluating patients by using an in vitro assay for IgE directed against specific antigens. This test was first made available as a radioimmunoassay (Nalebuff et al, 1981). The antigens are permanently bound to solid-phase polysaccharides, and the serum of the patient is exposed to this solid-base reagent. Antibodies, including the IgE antibodies, bind to the antigen; and nonspecific antibodies are easily washed away from the solid phase. The solid phase is then exposed to radioiodinated, purified antihuman IgE antibody. After this has been allowed to react with any IgE present, it is again washed, and the amount of radioactivity that is bound to the solid phase can be detected. This test can detect IgE in the serum of patients without any in vivo testing, which is an important step forward in the assessment of allergic individuals.

With the use of limited amounts of antigen, antibody other than IgE that binds to the solid phase can also be quantitated. Assuming that circulating IgE (which is not bound to mast cells) corresponds to the amount that is bound to mast cells (and functions physiologically), one can quantitate the amount of IgE antibody that an individual makes to a given antigen and predict a clinically significant antigen-patient encounter.

Because this test uses radiolabeling and absorbs both the IgE and the anti-IgE bound to an antigen on a solid-phase matrix, it is referred to as a *radio-allergosorbent test (RAST)* and diagrammed in Fig. 14-6. By doing a series of dilutions the RAST technique can give a profile for serum in the amount of bound IgE relative to appropriate positive and negative controls and can give a statistical probability about the individual's chance for clinical allergy to that antigen. Controversy arose over the end point for tests provided by two companies that first made these tests clinically available. This type of testing must be evaluated with the same statistical analysis that is applied to every clinical situation.

The physician wants to determine whether the patient's response is abnormal. In a test with primarily one end point, the higher the amount of serum IgE that is bound to a given antigen, the more the probability that the patient is clinically allergic to such an antigen. Before individual antigens are tested, a baseline total IgE level must be determined. Once this measurement has been made, the possibility of having no IgE response at all shifts this to essentially a single-tailed test. This statistical analysis shows that 1.64 standard deviations above the mean will yield a confidence level of 95%, predicting that the two tests in question belong to two separate groups. (That is, what is considered to be normal test results and the patient's test value come from a distinctly different population.)

Statistical predictability or confidence by which this can be predicted increases as one adds additional tests or clinical information. Physicians often forget this fact. The problem with most clinical evaluations is that there is some overlap between the normal and the

abnormal populations. Although statistically it may be quite easy to differentiate the two populations, individual responses of the two groups may overlap a great deal. All that can be offered in the evaluation of a given individual is the statistical probability that the individual falls into either the normal or the abnormal group. Fortunately, such findings can be confirmed by other in vivo testing.

This argument, however, should not detract from the significance of the RAST technique for the general population. Newer forms of the RAST technique have few false-negative and false-positive results if used properly. Control serum for the RAST technique is human umbilical cord blood, as it has been well established that fetuses do not make IgE. Proliferation of IgE-bearing B cells take place only after birth. The control defines a range of background of nonspecific bound radioactivity that may be encountered in a given test system.

Appropriate uses for the RAST technique have been enumerated (Nalebuff et al, 1981): patients not responding to conservative management, patients with relative or absolute contraindications for in vivo skin testing (including psychologic effects and discomfort involved with testing), patients unable to stop medication that adversely affects skin testing, patients who are not responding to their immunotherapy, and patients with IgE-mediated hypersensitivity to venoms, chemicals, and food. Inappropriate uses specific to the RAST technique include patients with total IgE levels below 10 U/mL, patients who have not had IgE levels determined at all and have not been examined by a physician ordering the test, and patients who have symptoms but negative skin test results.

Some consideration should also be made as to whether the evaluation of the patient's allergy will change the course of therapy. For instance, if a clinical diagnosis of allergy to environmental factors can be made and the patient can be treated with environmental control and antihistamines, further evaluation is probably not indicated unless the patient specifically requests it. In addition, a clinical diagnosis that involves a non-IgE-mediated allergic phenomenon, such as that associated with the intravenous administration of radiographic dyes, does not warrant further allergic workup unless the patient has extenuating symptoms (Patterson and Anderson, 1982).

After immunotherapy has been initiated, it is not possible to follow a patient's progress with the standard RAST technique because of the interference of IgG antibodies, which will compete for binding sites on the solid-phase medium. Radiolabeled antihuman IgG, IgM, and IgA antibodies can detect blocking antibodies.

Because of the problems of radioactive waste disposal, the principle of the RAST technique has been modified to use an enzyme-amplifying system instead of a direct radiolabeled reagent. In this system an enzyme that can cause a color change when exposed to a reagent is covalently coupled to the anti-IgE antibody. Once the system has been washed, it can be allowed to react with the reagent in the developer, and the amount is then measured in a colorimeter. If very high levels of binding are expected, the reaction time can be modified to keep it within a measurable range. Similarly, its sensitivity can be enhanced by increasing the reaction time. As this test involves an enzyme-linked immunoassay, it is called an ELISA test. An ELISA test that has been used clinically is the IgE fluoroallergosorbent test (FAST) technique, which uses a high-speed fluorometer (Tsay and Halpern, 1984). This

test also uses a monoclonal antibody to determine the IgE bound to the solid-phase medium. This method helps standardize reagents from batch to batch and, therefore, standardizes the results, which are measured over many years. The main advantage of the IgE FAST enzyme-linked analysis is that it does not require the licensing, monitoring, and disposal problems associated with radioactive materials; and it is currently faster than previous testing, results being available within 6 hours. With any test that has multiple stages, the sensitivity can be only as great as its most insensitive points (that is, the weakest link in the chain). Standardization of antigen and specific identification of human IgE are the critical steps to consider in evaluating the usefulness of such a test.

Allergy immunotherapy

Therapy aimed toward interrupting the immediate hypersensitivity reaction is aimed either at inhibiting or decreasing the probability of an antigen encountering an IgE molecule or at modifying the effects of chemical mediators (Ishizaka, 1982b). This can be achieved by a variety of mechanisms. Sometimes the individual must change his environment, which may involve relocation, a job change, and diet and pet restrictions. Antigen (allergen) avoidance is the simplest therapy. It requires specific antigen identification and removal, inactivation, or containment.

More complex is the theoretic activity of increasing antibodies of other classes that combine with the active sites of these antigens so that they are not available for IgE binding and subsequent reactions. This concept has given rise to the common clinical practice of desensitization whereby a given antigen is injected into an individual at levels below that which would cause an anaphylactoid type of response, with the hope that enough of the antigen will be available locally to stimulate antibody synthesis, probably of the IgG or IgA class. Obviously, a specific antigen must be identified and then administered. It is easy to imagine that IgA antibody, if secreted, could render inactive many antigens in the environment that are inhaled or ingested, keeping them from initiating a systemic reaction; however, most blocking antibody made after injection is IgG.

Even though hyposensitization has been available for almost 50 years, the exact nature of how this therapy benefits patients is not clear. The presumption has been that the formation of blocking antibodies, those which combine with the active sites, is responsible for this phenomenon. Recent studies with immunoassays for IgE and IgG antibodies, however, have not completely supported this concept. Patients sensitized to ragweed and begun on immunotherapy increase their IgG antibody titers 30 to 40-fold within the first 1 to 2 months. During this time, IgE antibody titers also rise significantly. Clinical improvement is seldom seen within the first month or so of treatment, and often only after years is "desensitization" observed. What has been observed is that after years of treatment, the IgE antibody level becomes significantly depressed and is below the pretreatment level in many patients. It also has been noted that the secondary response to an antigen by IgE is significantly depressed in patients who have been desensitized.

One must add to the observations that accompany the success of desensitization therapy both a gradual decrease in circulating IgE antibody levels and a suppression of the secondary IgE antibody response, in addition to an increased specific IgG antibody titer. Evidence suggests that the increased levels of IgG are not responsible for the suppression of

the secondary antibody response. The decrease in IgE probably is a result of some change in the memory cell populations. It has been speculated that the T cells, which have a regulatory role in the antibody response, change in some way that has yet to be identified. Evidence supporting this speculation has identified that the helper function associated with antigen-priming B cells diminishes with weekly injection of antigen. Induction of antigen-specific suppressor T cells by desensitization probably regulates the antibody response to the naturally occurring antigen.

Modes of therapy aimed at inhibiting the action of the agents released from the mast cell during degranulation do not require identification of specific antigens. The action of the antihistamines is obvious. The action of prostaglandin synthetase inhibitors inhibits the formation of the prostaglandins and thromboxanes, and the steroids inhibit the membrane phospholipid catalysis to arachidonic acid, which is the substrate for prostaglandin synthetase. Smooth muscle contraction can be relaxed with a bronchodilator, and alpha-adrenergic agonist increased secretion can be treated with agents that provide a cholinergic blockade or decreased blood flow to the affected areas. Sodium chromoglycate appears to stabilize mast cells and prevent them from degranulating, despite the formation of IgE antigen complex (Marks, 1984).

Tumor Immunology

A discussion of tumor immunology pertaining to the head and neck must first begin with a discussion of the antigens that we know are involved in this process. Tumors have been shown to have numerous surface antigens that are distinct from the supporting cells (Kirkpatrick and Fahey, 1982; Wanebo, 1979), including specific tumor antigens, virus receptors, HLA determinants, and for certain gut-associated tumorous antigens, a chorioembryonic antigen. Patients do indeed make antibodies to their tumors, as has been observed for many years. Carey et al (1983) followed a patient's antibody status over 1 year and were able to grow three separate cultures of tumor cells from the patient. Investigation showed that although the patient had an initial antibody titer of 1:128 against the tumor, this titer declined to undetectable levels over the next 6 months as the patient was treated.

A study of the antigen phenotypes on the surface of squamous cell carcinomas shows that the carcinomas may have appropriate blood groups and $beta_2$-microglobulin and will show antigens of a differentiated squamous epithelium because they are positive when stained with antibodies made by patients who have pemphigus. Monoclonal antibodies ("A 9" and "E 48") have been raised against squamous cell carcinomas in culture, and some of them will cross-react with many squamous cell carcinoma cell lines, as well as with some nonmalignant squamous cells (Carey et al, 1983; Quak et al, 1990) often staining the proliferating basal cell layer. In addition to the antigens that may be elicited in an antigen-antibody response, other antigens on other cells are important to consider, particularly those of the leukocyte antigens associated with the immune response, such as the class I MHC antigen, Ia, in rodents and its counterpart in humans, HLA-DR. HLA antigens are associated with an increased incidence of certain tumors. In addition to those antigens for Hodgkin's disease. Sin 2a antigens are found with 100% frequency in patients with nasopharyngeal carcinomas.

The antibody molecules are discussed earlier in the section on infectious diseases but need to be reconsidered here as they pertain to the immunologic response to tumors. It is

important to note that the Fc portion of the IgG molecule is quite distinct from the corresponding portion of IgA, as IgA has no activity to fix complement, activate phagocytosis, or encourage emigration of inflammatory cells. It is easy to imagine how a lymphocyte "feeling its way" around a tumor cell blanketed with IgA gets a much different chemical picture than if it were covered with IgG. This consideration is of importance in a discussion of the virus-associated antibodies observed in patients with squamous cell carcinomas of the head and neck. As noted earlier, antibody to tumors does not necessarily confirm any degree of immunologic containment. In fact, antibody may wall off the tumor from other immunologic processes, and "blocking antibody" may change the surface of the tumor so that it is not recognized as an area of inflammation. Other processes, enumerated in the following list, are discussed throughout this section.

Possible immunologic processes involved in tumor protection

Antibody coating (walling off)
Blocking antibody (IgA)
Circulating immune complexes
Suppressor T cells
Suppressive surface antigens I-C and J-I (MHC II?)
Prostaglandin inhibition of lymphokine action
Cytokine effects (cachexia)
Fibrin deposition by macrophages (walling off)
Low molecular weight macrophage inhibitors
Antigenic modulation of antigen-complement spatial relationships
Histamine activation of suppressor T cells
Neovascular (signal recognition, altered perfusion)
Iatrogenic effects (immunosuppressive chemotherapy).

The understanding of tumor biology is far from complete, but many of the factors that enter into the interaction are known. The interactions that are the body's antitumor energy and the counterforce, the absence of energy, tumor anergy, are diagrammed in Fig. 14-7. First consider antitumor energy. Some tumor cells are observed by the immune system to have special surface antigens and are processed through the T lymphocyte system to produce cytokines, which both promote antitumor effects (cytostasis IFNs-alpha, beta, gamma and cytolysis IFN-gamma, TNF-alpha) and activate antitumor cells (that is, Il-1: macrophages; Il-2: T-lymphocytes; Il-4: B lymphocytes; INF-gamma, TNF-alpha: macrophages).

The tumor cells may also be processed by other accessory cells such as macrophages or dendritic cells (Fig. 14-7, upper right) to stimulate production of antibodies, which may be directly cytotoxic or may participate in antibody-directed cellular cytotoxicity (ADCC). The squamous cell carcinoma (SCC) cells arise from cells that are normally adherent, and cytokines or other differentiating agent such as retinoids should inhibit their migration and therefore discourage metastasis. We know little about the other phagocytic cells such as polymorphonuclear cells (PMNs) and eosinophiles except that the infiltration of eosinophils has been associated with an increased patient survival (Goldsmith). Recent evidence from Huber et al (1991) indicates that neutrophil movement out of the bloodstream and into an area of inflammation is directed by the production of interleukin-8, which up-regulates the expression of leukocyte, integrins and the shedding of neutrophil lectin adhesion molecule-1.

The other perspective is one of the tumor's immunosuppressing factors, which gives the cancer an advantage. SCC has been shown to produce several factors directly that can inhibit the immune response. Tumor growth factor beta (TGF-beta) inhibits the production of surface (adhesion) molecules. Prostaglandin inhibits the production (but not the function) of lymphokine-activated killer (LAK) lymphocytes and may affect other aspects of the immune response also. SCC produces IL-1, which is involved in bone resorption breaking physical barriers to metastasis. Altered adhesion and intrinsic motility of cells may also contribute to a tumor's potential for metastasis. The tumor cell may hide antigens by appearing as an antigen-processing cell itself by expressing MHC II on its surface and thereby discourage the presence of other antigen-processing cells. SCC may simply appear as a normal cell with no unique surface antigens. This fact is borne out by the magnitude of effort to produce monoclonal antibodies to unique SCC surface antigens with very few positive exceptions (Carey et al, 1983; Quak et al, 1990). The antibodies produced may combine with antigens that are shed into the circulation in quantities small enough so that soluble complexes form, which further immunosuppress the host. Tumor cells may encourage the production of antibody production of subtypes that do not effectively stimulate an immune response (ie, IgA). The immune system may inadvertently provide an advantage to the tumor by liberating histamine in an initial inflammatory response that induces a lymphocyte suppressor substance, which activates suppressor lymphocytes without increasing their number. The production of TNF-alpha may not be effective in killing SCC cells but may instead cause the systemic response of cachexia of chronic disease for which it was co-discovered and named "cachectin".

It is impossible to show all the possible interactions of tumors and the immune system here. Fig. 14-7 demonstrates how complex the area of study has become and how difficult it is to isolate one aspect of the immune system for testing.

Viruses and cancer

The discussion of tumor immunology must include the concept of virus-related neoplasia and oncogenes (Weinberg, 1983). The tumor model proposed by Vogelstein (& Vogelstein) for colon carcinoma requires three genetic mistakes for complete malignant transformation: (1) an increase in oncogene expression, (2) an error in p53 (a mitosis suppressing protein), and (3) cell adhesion receptor effect (contact inhibition). Research has failed to show a consistent elevation in a single oncogene for SCC. Nevertheless, a virus of great interest is the papilloma virus group, which includes the causative agents of laryngeal papilloma (Bransdma and Abramson, 1989). These viruses are the etiologic agents of laryngeal papilloma, and indeed their complementary DNA can be extracted from tissues of patients with this disease.

Attempts to make vaccines from fractions of these tissues has met with limited success and in recent years has been replaced by immunotherapy with interferon (Kashima, 1991), which is discussed later in this section. DNA from papilloma virus has been found in verrucous carcinoma tissues, and a small number of SCC specimens, implicating this virus as having true oncologic potential. Recent evaluations reveal that gene products of human papilloma virus (HPV) bind to and inactivate p53 in normal cells and, therefore, could initiate changes in the mitotic potential of infected cells.

Before interest in HPV, investigators were interested in antibody to herpes simplex virus (HSV) tumor-associated antigen, which was found in 85 of 93 patients with squamous carcinomas of the head and neck (Wanebo, 1979). Of interest is that IgA antibody titers to HSV antigens also are increased in similar patient populations and are increased in heavy smokers in contrast to controls. Since IgA antibody can bind to antigen sites but does not initiate leukocyte-associated immunologic responses, this effect could be a protective one (from the perspective of the tumor). This consideration is not trivial, as HSV has been shown to induce squamous cell carcinoma in mice when used in conjunction with ultraviolet exposure and local application of a tumor promoter (TPA) (Burns and Murray, 1981).

Another virus that is intimately associated with head and neck cancer is the Epstein-Barr virus (EBV). There is strong evidence of its association with Burkitt's lymphoma and nasopharyngeal carcinoma (Henle and Henle, 1976; Neel at al, 1983). This virus is of considerable interest, as it seems to induce an energy-like state, which can be observed in patients with acute infectious mononucleosis (Kantor, 1975; Kantor and Siegal, 1979). Lymphocytes taken from patients with acute infectious mononucleosis stimulated by mitogens or antigens have an abnormally low mitogenic response, and it takes as long as 40 weeks for the response to return to normal. The immunosuppression probably occurs through the T-lymphocyte's attempt to hold the EBV-driven B cell proliferation through its natural control mechanisms. The only part of the immune system that can be suppressed is the normal part (which is not the problem), and clinical immunosuppression occurs. This factor could contribute to the propagation of some tumors, given the proper sequence of events. There is good evidence that the DNA of EBV is found in nasopharyngeal carcinoma, but evidence for a specific viral agent as the initiating agent of SCC is lacking.

Cell-mediated immunity

If one examines the peripheral blood, separated by buoyant density, of an average individual (Fig. 14-8), one can separate white blood cells into two groups: polymorphonuclear leukocytes and mononuclear cells. Mononuclear cells are made of approximately 70% lymphocytes and 30% monocytes. The monocytes can be identified morphologically and by their phagocytic activity, which can be measured in a laboratory by allowing these cells to engulf, for example, latex particles. The lymphocytes can then be subdivided into three basic categories of cells. About 10% to 15% of the cells have surface IgG and are designated as B cells. These cells have been designated as B cells in mammals in recognition of the bursa of Fabricius, which is present in fowl and is in part responsible for the maturation of this cell type. The surface immunoglobulin is not attached to an Fc receptor, but its mode of attachment is not clear. These cells manufacture the antibodies that are on their surface and can belong to any of the immunoglobulin subtypes described previously. In addition to the surface immunoglobulin, cells have Fc receptors that can allow immunoglobulin molecules bound in an Ab-Ag reaction to adhere to their surfaces. The binding constant differential for these two means of attaching immunoglobulins to the surface of cells differ by several orders of magnitude, the surface immunoglobulin being much more tightly bound. The B cells are not distinguishable morphologically from the other lymphocytes but can be readily identified by staining with antibodies to immunoglobulins, showing that they indeed have the antibodies on the surface membranes. In contrast to the B cells, T cells and null cells, which are the other two groups, do not have surface immunoglobulin, but they may have Fc receptors that can allow them to identify antibody complexes. The different cells are currently identified by

surface cluster of differentiation (CD) antigens usually with fluoresceine-activated cell sorter (FACS) or immunohistochemistry (Table 14-1).

T cells are the largest subset and make up about 80% of the peripheral blood lymphocyte population. They can easily be identified by noting how many of the cells form rosettes when placed in culture with sheep erythrocytes. The sheep erythrocytes adhere to the surface of the T cells, and small clusters of cells can then be observed, allowing one to calculate the percentage of rosetting cells or T cells. Of the T cells, about 55% to 65% are helper T cells and have the surface antigens identified by monoclonal antibodies to T1, T3, and T4. Twenty percent to 30% are suppressor T cells or cytotoxic T cells; they contain T1, T3, and Ts or T8 cells and are involved in B- and T-cell suppression (Rosen et al, 1984). Helper T cells are responsible for stimulating B-cell immunoglobulin production and both B- and T-cell proliferation. The T cells are so named because they depend on the thymus for their maturation and are not present in patients or animals with thymic aplasia. They produce lymphokines in response to a variety of stimuli.

T cells

T cells are important for many reasons. One of their dramatic features is their early recognition of foreign antigens present on the surface of accessory cells, such as macrophages, dendritic cells, and Langerhans cells. T cells are the lymphocytes responsible for the production of soluble protein messages called *lymphokines,* which produce a multitude of effects. The lymphokines produced by lymphocytes and cytokines produced by macrophages and other immune cells control the growth and differentiation of many immunologically active cells. Cells are thought by many to begin differentiation from a pluripotent stem cell, which can also give rise to other lymphocytes. This process begins early in embryonic life and involves the relationship between the ectodermal cells of the third and fourth pharyngeal pouches and the mesenchymal cells of the pluripotent stem cell (Rosen et al, 1984).

Ectodermal cells continue to grow in the thymus and pursue their migration into the mediastinum. First, undifferentiated cells entering the thymus appear as early as the eighth week of gestation, and the glandular tissue quickly becomes filled with lymphoid cells. When the cells enter the outer cortex, they are induced to become thymocytes and begin cell division. As the cells grow, this dividing population emigrates toward the medullary regions; the cells then cease to divide and undergo maturation. Both the growth and maturation occurs under the influence of soluble factors, such as thymosin and thymopoietin, which are secreted by the thymic epithelial cells. These hormones are produced in greater quantities in younger individuals until they decline rather abruptly, often in the third decade of life.

After their passage through the thymus, cells find a variety of tissues, determined partially by the T-cell surface receptors and complementary determinants present on the endothelial cell membranes of lymph nodes or other lymphoid tissues. Cells then migrate to the paracortical regions of lymph nodes or to complementary sites in the spleen, intestinal lymphoid and viscus-associated lymphoid tissues. After they initially enter this circulation, cells acquire their T lymphocyte markers (T_1, T_3, T_4, T_5, and T_8) as mentioned earlier. Once in the target lymphoid organ, the T cells stay only a relatively short time before reentering the circulation. While there, however, they are capable of exerting an enormous influence on the other lymphocytes. When helper T cells interact with both complementary antigen and the

HLA-DR determinant, the antigen-presenting cell, they are able to undergo blast transformation and cell division. It is the binding to these surface determinants, mimicked by phytohemagglutinin A, and pokeweed mitogen, that induces this proliferation. Accessory cells, such as dendritic cells, Langerhans cells, or macrophages, are required for activation and may produce interleukin I (IL-1), which in turn induces the helper T cells to produce interleukin 2 (IL-2), a T-cell growth factor (Dinarello, 1984). Activated T cells also express both HLA-DR and surface receptors for IL-2. Cytotoxic T cells are induced to exert effects (Table 14-2) in a similar manner after contact with an appropriate antigen and HLA determinant but need the presence of IL-2 to be converted to active cytotoxic T cells. In this way, suppressor T cells depend on helper T cells for activation. Helper T cells activate proliferation of subsets of T and B cells; suppressor T cells suppress. Subgroups of both helper and suppressor cells are being defined. Activated T cells secrete lymphokines, including macrophage chemotactic factor, interferon-gamma (INF-gamma) (which also has a macrophage-activating factor), and growth factors for B-cell growth and differentiation.

An additional mechanism by which T and B cells are controlled is by activation of suppressor T cells by histamine. This action can be blocked by cimetidine, indicating a type-2 histamine receptor. Anergy association with chronic mucocutaneous candidiasis can be reversed in vivo with cimetidine (Jorizzo et al, 1980) and may also be active in tumor-associated anergy (Fig. 14-9). Although skin test anergy and cellular immune responses can be reversed in head and neck SCC patients with H2 antagonists (Richtsmeier et al, 1987a) the role that has in clinical tumor progression is unknown.

The final subgroup (the null cells) accounts for 1% to 10% of the cells and may have both complement and Fc receptors. Within this group of cells is the natural killer (NK) cells, which seek out and kill both tumor cells and host cells that are chronically infected with viruses, such as EBV-transformed tumor cells (Heberman, 1982). Unfortunately, the spectrum of tumor cells susceptible to NK cell killing is quite small, principally lymphoic cells. Other designations for this population of cells have used "K cells" and the "third population". Basically, they are identified as having none of the properties attributed to the other two groups, except for the presence of Fc receptors.

It is likely that all peripheral B cells bear complement receptors. Those found in lymphoproliferative tissues, such as those in Waldeyer's ring, may not be complement receptor positive because of immaturity. The class II MHC surface antigen production can be stimulated in B cells in the face of an inflammatory response and as the B cells mature. (Class II MHC may also be present constituently on accessory cells: macrophages, dendritic cells, and Langerhans cells.)

Cellular hypersensitivity and cytotoxicity can be mediated by the cells described previously, the most ominous-sounding activity being the NK activity of the null cells as they come in direct contact with virus-infected or tumor cells. T cells can identify a local inflammatory area by the lymphokine released, again inducing the release of soluble toxins promoting phagocytosis, releasing enzymes into the area, and killing cells by direct contact. Sensitized T cells can also promote a cytotoxic effect against cells to which they have previously been exposed when they come in direct contact with such cells.

B cells, on the other hand, are basically the antibody-forming mechanism of the body; and their participation in hypersensitivity involves complement and complement-mediated lysis or antigen-antibody-mediated activity that attracts NK cells or other inflammatory cells, resulting in the generalized inflammatory response described in basic pathology texts. Tumor cells can then be killed by cytotoxic (that is, killer) T cells, NK cells, and macrophages and monocytes that can phagocytize them. B cells promote antibody production, which lyses cells in cooperation with complement. The T-cell lymphokines, such as macrophage-activating factor (MAF), macrophage inhibitory factor (MIF), IFN-alpha, IFN-beta, and IL-2, can either recruit other cells to the area or have direct antitumor effect.

Table 14-1. Cluster of differentiation (CD) classifications for selected leukocyte subset identification

CD Ag	Name	Cellular subsets/function
CD1a		Thyrocytes
CD2		T-lymphocytes, NK cells, thymocytes
CD3		T-lymhocyte antigen receptor
CD4		Helper T-lymphocytes
CD6	"Pan T Cell"	Most (90%) T-lymphocytes
CD8		Cytotoxic/suppressor T-lymphocytes, NK cells
CD11a	"LFA-1"	Leukocyte adhesion antigen
CD11b	"Complement receptor"	T-cells, monocytes, granulocytes
CD11c	"Mac-1"	Monocyte/macrophage
CD16	"Fc receptor" for IgG (type II)	NK cells
CD19	"Pan B Cell"	B-lymphocytes (early)
CD22	"Pan B Cell"	B cell differentiation Ag (late)
CD24		Pre-B cells
CD25	"TAC-1"	T and B-lymphocytes, large granular lymphocytes, IL-2 receptor
CD32	"Fc receptor" for IgG (type I)	
CD45R	"common leukocyte Ag"	T and B-lymphocytes, NK cells, granulocytes
CD54	"ICAM-1"	Activated lymphocytes, macrophages
CD56	"rhinovirus receptor"	NK cells.

The lymphokines are regulatory proteins, as can be seen from their names. They probably function most dramatically to regulate the immune response but have effects on other cells as well, such as the cytostatic and cytolytic effects of IFN-gamma. The interleukins are important in augmentation of the T-lymphocyte functions, and interleukin 3 (IL-3) plays a role in the formation of cytotoxic T cells. The potential for these lymphokines and others, such as tumor necrosis factor and lymphotoxin, to be clinically useful has initiated serious investment by private industry. Presumably, industry will involve itself with the same enthusiasm that was present for the interferons, the first lymphokines to be genetically cloned (Panem, 1983).

The interferons were the first cytokines identified and the first cloned and, therefore, serve as a model for the investigation of other cytokines. In addition, there are multiple reports about the effect of interferon on laryngeal papillomas (Leventhal et al, 1991;

Richtsmeier, 1984b). The exact antitumor mechanism of IFN's action here is unclear, as interferon itself has the same effect on the growth of laryngeal papilloma cells in culture as it does on normal epithelial cells. Which of the many effects of interferon on the immune system is responsible for its clinical benefit is unclear. IFN-alpha and IFN-beta have a very weak suppressive effect on the growth of squamous cell carcinomas in culture, but there are many other ways that IFN-alpha could help the patient with cancer through IFN-alpha/beta effect on the immune system. A single case report of an individual with an intracranial nasopharyngeal carcinoma treated with natural polyclonal IFN-alpha who responded dramatically has not been followed up with subsequent reports using recombinant IFN-alpha and may remain as an isolated case (Treuner et al, 1980). IFN-gamma has a more dramatic direct effect on SCC both in vitro and in vivo in the limited series reported (Richtsmeier, 1988; Richtsmeier et al, 1990).

Table 14-2. Cytokines involved in the immune response to (head and neck) cancer

Cytokine	**Cell sources**	**Immunologic effects**
IL-1	Macrophages dendritic cells epithelial cells	Lymphocytes activation/proliferation, induces other cytokines Increase NK activity, increase lymphokin productin, bone resorption
IL-2	Activated T-lymphocytes	Increase Tc activity, LAK induction, NK activity, lymphokine production, macrophage activation, induces IFN-gamma and TNF-alpha
IL-4	Activated T-lymphocytes	B cell proliferation, immunoglobulin production, MHC II/FcR expression, macrophage activation, IgE switch and mast cell growth
IL-6	Fibroblasts	B cell growth factor, polyclonal Ig production
IL-8	Macrophages	Granulocytes chemoattractant
TNF-alpha	Macrophages	Tumor necrosis/cytotoxicity, vascular permeability, lipid metabolism
	Lymphocytes	

TNF-beta
- Endothelium
  - Neutrophilia, increase lymphokine production (TNF-beta = lymphotoxin)
- Keratinocytes

TGFalpha
- Lymphocytes
  - Inhibit lymphocyte proliferation
- Macrophages
  - Suppress NK/LAK activity
- Platelets
- Keratinocytes
  - Combines with EGFr to induce keratinocyte growth

TGFbeta
- Keratinocytes
  - Suppresses response to cytokine, fibroplasia
- Platelets

INF-alpha
- Leukocytes
  - Antiviral, cytostatic, augments lymphokines, MHC I

INF-beta
- Fibroblasts
  - Cytostatic, cell differentiation, MHC I expression

INF-gamma
- T-lymphocytes
  - Cytotoxic, cytostatic, increase NK activity, MHC I and II expression, CTL activation, increase lymphokine (including self) production, cell differentiation

G-CSF
- Monocytes
  - Neutrophil growth factor

M-CSF
- Monocytes
  - Macrophage growth factor

GM-CSF
- T-lymphocytes
  - Mononuclear and granulocyte growth factor.

The interferons have been divided into three distinct subclasses acknowledged by the World Health Organization as IFN-alpha, interferon-beta(IFN-beta), and IFN-gamma. IFN-alpha, previously referred to as "type 1 interferon" or "leukocyte interferon", is routinely

produced by lymphocytes and response to virus exposure. Lymphoid tissues of Waldeyer's ring have been shown to be a rich source of interferon production when such tissues are removed because of recurrent infection stimulated in the laboratory. The clinical significance of removing these tissues is unknown at this time. IFN-alpha is at least as stable to acid treatment as are the immunoglobulins. There are at least a dozen immunologically distinct subtypes of human IFN-alpha (HUIFN-alpha). IFN-beta is generally thought to be produced by the connective tissue cells exposed to viruses, nucleic acids, or endotoxin and is also quite acid stable. In vivo virus treatment produces a mixture of acid-stable interferons (IFN-alpha/beta). IFN-alpha and IFN-beta share the same receptor so that similar effects might be expected. Despite the common receptor, IFN-beta has a more dramatic differentiating effect in some systems. Thus, the IFN-beta-receptor complex that results and moves into the cell to trigger cellular responses must have a different conformation than the IFN-alpha-receptor complex. The material referred to as IFN-beta2 has been renamed IL-6, as it has little antiviral action and a much more important lymphocyte regulatory function.

In contrast to IFN-alpha and IFN-beta, IFN-gamma is much less stable. Previously referred to as "immune interferon" or "type 2 interferon", this protein is produced by T lymphocytes in response to antigenic stimuli and a variety of other stimuli that cause T-cell proliferation. Under most circumstances, IFN-gamma can be distinguished from the other interferons by its instability during acid or mild sodium dodecyl sulfate treatment. An unusual acid-unstable IFN-alpha has been observed in the serum of patients with autoimmune disorders (rheumatoid arthritis, Sjögren's syndrome) and acquired immunodeficiency syndrome (AIDS).

The interferons have a remarkably broad range of inducers and stimuli. Many of the effects are immunologically important (Stiehm, 1982). These effects include the acquisition of antiviral resistance, modulation of prostaglandin synthesis and (HLA) surface determinants, and suppression or enhancement of the antibody production (depending on some specific circumstances of the test system). They inhibit pinocytosis and cell growth, including that of the bone marrow. Interferon has an effect on the recruitment of NK cells in the granular lymphocyte pool, but the results are variable in different clinical settings. Macrophage activation has been attributed to IFN-gamma, which encompasses a profound effect on the primary scavenger cell system of the body (Spitalny and Havell, 1984; Svedersky et al, 1984). Interferon has been shown to have additive effects with other chemotherapeutic agents and immunomodifiers, such as H_2-histamine antagonists and prostaglandin synthesis inhibitors.

The interferons are able to exert their antiviral factors through a variety of mechanisms (Richtsmeier, 1984a, 1984b). They have an effect on virus cell attachment, uncoding, transcription, translation, and assembly. The cell becomes resistant to viral infection through an active process that includes synthesis of new RNA and protein. Among the proteins produced is an enzyme 2',5'-oligoisoadenylate synthetase, which produces a unique peptide, 2', 5'-oligoedenglate (2',5'A). 2',5'A is similar to oligonucleotides of the RNA series containing only adenosine bases; however, the linkage is 2-5 rather than 3-5. 2'-5'A appears to have antiviral effect directly and also activates an endonuclease that is specific for some viral RNAs. The exact mechanism by which interferon exerts its antigrowth effect appears to be at least as complex as the molecular sequence involved in the acquisition of antiviral resistance (Gresser, 1982). Some of the areas of interferon action are listed as follows:

Potential interferon antitumor effects
Growth inhibition
Cell growth
Cell proliferation
Postmitotic accumulation in Go
Decreased DNA polymerase
Decreased Go to G, transition
Thymidine transport and phosphorylation
Cell differentiation
Cytolytic activity
Inverse to interferon sensitivity
Immunologic effects
NK cell recruitment
Suppressor T-cell induction
Antibody synthesis
Prostaglandin synthesis
Antiviral effect
Antipapillomavirus
Anti-EBV.

The side effects of parenteral IFNs can be substantiated (neuropsychiatric changes, bone marrow suppression, liver toxicity), and although topical interferon is effective as used in the nose, its use is limited (Hayden and Gwaltney, 1983; Hayden et al, 1983). The monocytes can also liberate toxic compounds referred to as *monokines*, such as tumor necrosis factor (TNFalpha and beta), lymphocyte-activating factor (LAF), colony-stimulating factor (CSF), and interferons. They can release pyrogens and stimulate membrane changes in response to molecular changes, including those of the HLA determinants, as well as increase cytotoxicity.

By examining the lymphocytes taken from the lymph nodes of patients with squamous cell carcinomas of the head and neck, Houck et al (1983) investigated the ability of the host to respond to a tumor. Cells taken from patients with T_1 and T_2 lesions showed little change from control when stimulated with mitogens, as opposed to the situation with T_3 and T_4 lesions, which showed a broad range of increased activity. Similarly, little change from control was observed after stimulation of cells from well-differentiated tissues as opposed to a 1.5 to 2.5-fold increase in activity after stimulation of cells from patients with moderate to poor grades of differentiation. Other observers (Richtsmeier 1991, Mekel) have noted regional immunosuppression of lymphocyte functions in neck node lymphocytes.

Macrophages and accessory cells

The last subgroup of cells that merits discussion is the macrophage group. Macrophages have long been known as the "garbage cell" of the immune system and have been responsible for initiation of a number of inflammatory processes, including antigen presentation and IL-1 production. They are certainly important in tumor immunology (Cameron and Stromberg, 1984). Interest has been aroused over the identification of an accessory cell activity in skin (Langerhans cells) and lymphoid (dendritic cells), which does not use the macrophage as an accessory cell (Thiele and Lipsky, 1982; Thiele et al, 1983).

Beginning in 1973, Steinman and his colleagues at Rockefeller University described a novel cell in the peripheral lymphoid organs of mice (Steinman and Nussenzweig, 1980). These cells are referred to as *dendritic cells* because of their irregularly shaped dendritic processes but are bone marrow-derived leukocytes and are not part of the nervous system. The general group of dendritic cells are leukocytes referred to in other works under a variety of names, including interdigitating cells (as well as dendritic cells) in lymphoid tissues and veiled cells in lymph (Van Voorbis et al, 1983). These are similar to but can be differentiated from Langerhans epidermal cells and follicular germinal center dendritic cells.

The recent major effort has been to distinguish dendritic cells from cells within the macrophage group (Parwaresch et al, 1983). Dendritic cells lack active endocytic capacities, the presence of which is a cardinal feature of other mononuclear cells (Tew et al, 1980; Van Voorhis et al, 1983). They also lack Fc receptors that could help mediate phagocytosis. Both dendritic cells and macrophages are leukocytes that lack lymphocyte differentiation and surface antigens but retain the major histocompatibility complex antigens. Accessory cells must express class II MHC next to the processed Ag on their surface for lymphocytes to recognize the Ag. Human dendritic cells differ from Langerhans cells in that they lack Birbeck granules and do not express the OKT-6 antigen, whereas rat dendritic cells contain Birbeck granules (Haines et al, 1983; Van Voorhis et al, 1982, 1983). Langerhans' cells have been identified in cholesteatomas (Veldman et al, 1984b). These cells appear to be a central part of the immunologic response mechanism in that they are accessory cells for the mixed lymphocyte response in mice (Steinman et al, 1983) and humans (Crow and Kunkel, 1982), which may in part be mediated by IL-2 dependent mechanism in both mice (Riccardi et al, 1983) and rats (Klinkert et al, 1980; Wong et al, 1982). Physiologic measurements of dendritic cells have been reported for human head and neck lymphoid tissues, where they have been found to be necessary for Ag stimulated IFN-gamma production.

Immunologic tumor cell killing

The variety of immunologic phenomena that can be recruited to kill tumor cells is quite diverse, but can be divided into basically two types: those that are cell-directed and those that are basically molecular phenomena that directly affect the tumors or recruit other cellular phenomena (Table 14-3). On the cellular side, an activity that was thought to be the most important in the routine surveillance for tumor cells is that associated with NK cell activity, although now few tumor cells are known to be susceptible to NK cells. Specific tumor cells can be killed by cytotoxic T cells, and, of course, macrophages and monocytes are important in tumor processing; but actual antitumor effects of macrophages are not as well documented as for the preceding two groups of cells (see also, the discussion of tumor immunotherapy). Complement receptor-positive B cells can help direct cytolytic activity, and polymorphonuclear leukocytes have been shown to contribute to at least a cytostatic antitumor effect.

On the molecular side, IgG can mediate cell lysis with complement and recruit cells associated with antibody-dependent, cellular cytotoxicity. The antigen must be exposed to the circulation both for recognition and for adequate antibody delivery. The permeability of the tumor neovascularization has been useful and important in delivering antibody-tagged radionucleotide radiation to hepatomas in humans. Early work with a monoclonal antibody (Quak et al, 1990) shows great promise in imaging human SCC. Because the Ag is expressed

to some extent on normal proliferating basal cells, the specificity must be due partially to increased binding constant and partially to the increased escape of the antibody through the endothelium in the tumor bed into the extracellular space. IgG cell lytic effects are well identified. T-cell lymphokines include MIF, IFN-alpha, IFN-gamma, and IL-2. Monokines produces include LAF, CSF, IL-1, IFN-alpha, and pyrogens. In addition, membrane changes can occur in response to the previously mentioned chemical mediators (that is, interferon). Among these changes is an increase of HLA-DR in normal cells as part of the activation process. To demonstrate how these activities can exert a concerted effort on tumor cells, Fig. 14-9 demonstrates how these phenomena may interact. Many of these functions are discussed earlier in the text. An effort is made here to unify these concepts.

Table 14-3. Immunology of tumor cell killing

Cellular	**Molecular**
NK cells	IgG (ADCC) with or without complement
Cytotoxic (killer T cells)	
Lymphokine activated killer (LAK) cells	IgE
	Complement
Tumor infiltrating lymphocytes (TILs)	T-cell lymphokines, IL-2, IFN-alpha, IFN-gamma
Macrophages and monocytes	Monocyte monokines IL-1, TNF-alpha, pyrogens
CR and beta-cells	HLA expression
Antibody-dependent cellular cytotoxicity	

The center of Fig. 14-9 represents a tumor cell that is being acted on by four cellular components. These activities are in turn influenced by soluble mediators that come primarily from T cells. Among the most important are the lymphokines, which have activities exerted directly on the tumor, as well as the effects of mononuclear cells and B-cells. The production of lymphokines can be negatively influenced by tumor prostaglandins. Whether these activities occur sequentially or simultaneously is unknown. The answer depends, in part, on the location of the tumor and the immunologic status of the host.

Evaluation of immune competence in patients with neoplastic disease

Although evaluation of immune competence is discussed in greater detail in the section on immune deficiency states, it merits discussion here because of its prognostic potential. A history of frequent recent infections may provide a clue to the patient's underlying problems. Humoral immunity can be evaluated by serum immunoglobulin levels (with individual components separated by electrophoresis) and a count of the percentage of B cells in the patient's peripheral blood.

Cell-mediated immunity can be identified in numerous ways. Patients with head and neck tumors are deficient in total T cells (Olkowski and Wilkins, 1975). The most straightforward in vivo test is skin testing with antigens in which the patient has been previously exposed. Statistically significant tests include those for mumps, *Candida*, streptokinase, and paraphenylenediamine (PPD) (if the patient has previously tested PPD positive). A knowledge of the preillness status of these tests and the ability of these patients to respond to these antigens depend on their previous exposure. It is assumed that virtually

all patients should test positive to one or more of the antigens. Head and neck cancer patients have deficiencies in this aspect of cell-mediated immunity (Ellber et al, 1974; Wanebo et al, 1975). If the test results are negative, however, the ability of the patient to respond to a new exposure must be tested, as it is possible that a given patient has not previously experienced the antigens mentioned earlier. The most common test is to treat the patient with dinitrochlorobenzene (DNCB). Exposure to this chemical causes the individual to form antibodies to DNCB haptenes on host antigens, and the secondary challenge identifies whether the patient has responded. Similar immunization can be carried out with BCG or other etiologic agents. Patients who are DNCB-positive perform significantly better than do their counterparts who are DNCB negative at the time of their initial diagnosis and therapy (Mandel and Kiehn, 1974). The reason patients become anergic is not clear. Numerous mechanisms are evoked in different circumstances. Virus antigens may be involved, as mentioned earlier, which have been identified for EBV (Dwyer, 1984). The T-lymphocyte numbers, particularly the helper/suppressor T-cell ratios, may be important to understand a patient's response to the tumor. This measurement is particularly important in assessing the regional immunity. Unknown factors that change the helper/suppressor T-cell ratio may influence this response.

Immunologic phenomena that may be involved in "tumor protection" include antibody coating of the tumor with immunologic isolation similar to but distinct from fibrin deposition by macrophages, which also help wall off such a tumor, preventing antigen exposure and an effective immunologic response. Analysis of T-cell subsets has not yet provided an insight into the source of poor prognosis (Johnson et al, 1984). Blocking antibody of the IgA class may prevent adequate immunoglobulin-mediated immunity. Suppressive surface antigens of the I-C, I-J class influence response by lymphocytes. Katz (1983) has correlated stage, age, and a serum immunoglobulin prognostic index (SIPI), which corresponds with outcome. The SIPI is essentially a measure of the relative amounts of IgA to IgD or IgE, with higher amounts of the former and lower amounts of the latter yielding the worst prognoses.

Negative effects of the prostaglandins and interferons may also play a role. Although exogenous interferon may be beneficial for patients and antibody to interferon has been linked to a poor prognostic outcome, low-grade interferon production is seen in the immunologic tolerance state of pregnancy and in low levels may actually be immunosuppressive.

Recently a low molecular-weight macrophage inhibitor was identified in extracts from squamous cell carcinomas of the head and neck (Strasnick et al, 1990) that is not prostaglandin, TGF-beta, or lipid. The mode of action of this substance is not clear. In addition, iatrogenic effects of chemotherapy, radiation therapy, and possibly surgery may contribute to the patient's inadequate immunologic response.

Prostaglandins and cancer

Prostaglandins are produced in every tissue of the body and are involved in a number of inflammatory-type responses. They are discussed in this section because of the regulatory control that they appear to have on the immune system and because of the interest that physicians have had in the prostaglandins highlighted by research on the effects of prostaglandins and squamous cell carcinomas (Goodwin and Webb, 1980; Karmali, 1983). Prostaglandins act as proinflammatory compounds. Prostaglandins E1 and E2 (PGE1 and

PGE2) can cause both local blood vessel dilatation and local increased vascular permeability. They potentiate both the action of histamine and bradykinin, causing pain, and the accumulation of edematous fluid in an area of inflammation.

The flood of available prostaglandin synthesis-inhibiting anti-inflammatory agents is another strong piece of clinical evidence of their involvement in significant inflammatory responses. Although prostaglandins are, in general, thought of as promoters of inflammatory response, they have a less well-recognized action as anti-inflammatory agents with regard to negative regulation and humoral and cellular immunity. In this regard they may play a more important role in tumor immunology. Administration of exogenous prostaglandins and stimulation of endogenous prostaglandins have been shown in separate sets of experiments to inhibit the plaque-forming (antibody-producing) cells, in contrast to the situation with animals similarly stimulated but also treated with indomethacin. Mouse tumors can have their growth reversed in vitro by prostaglandin synthesis inhibitors, and Blitzer and Huang (1983) have shown suppression of growth of epidermoid carcinomas in the palate of rats that had been treated with indomethacin. Prostaglandins inhibit the production of LAK cells and killer cells remove from SCC patients treated with indomethacin are more effective than those removed from control patients (Snyderman et al, 1990).

To explain these phenomena, in vitro studies have identified the release of prostaglandin into culture medium by subsets of T cells after phytohemagglutinin stimulation. Suppressor T cells appear to increase their activity by releasing a low molecular-weight peptide that is highly suppressive of both T-H and B-cell mitogenesis. In human peripheral blood the macrophage appears to be the predominant prostaglandin-producing cell, but in animals macrophages in addition to T and B cells can produce prostaglandins. Additional reports also implicate prostaglandin as being in control of nonproliferative T cell functions. Lymphokine production appears to be inhibited by prostaglandins and augmented by indomethacin. Inhibition of prostaglandin synthesis has been shown to increase natural and antibody-dependent cytotoxicity in a human in vitro system.

Evidence suggests that B cells may regulate the response to certain antigens by producing prostaglandins, as evidenced by an increase in the number of plaque-forming cells after stimulation and treatment with prostaglandin synthesis inhibitors, even when this is performed in a highly purified B-cell preparation (Goodwin and Webb, 1980). Cameron and Stromberg (1984) have provided evidence that macrophage cytotoxic activity, induced by interferon containing preparations, could be inhibited by a serum factor, which was sometimes reversible by indomethacin. However, the in vitro cytotoxic activity could also be suppressed by indomethacin in other patients and does not seem to be a constant feature in their assay system.

Prostaglandins are a group of compounds derived from fatty acids, which have in common a five-member carbon (cyclopentane) ring. They are derived from arachidonic acid for PGE2 and dihomo-y-linoleic acid for PGE1. The reactions are catalyzed by the enzymes cyclooxygenase, endoperoxide isomerase, and peroxidase. The three are collectively referred to as *prostaglandin synthetase* and constitute the major pathway. The second pathway is derived from membrane-associated lipoxygenases, which can form potent chemotactic compounds. Metabolism of the same substrate can be alternatively converted into a group of products called *leukotrienes.* Among the leukotrienes is the substance previously referred to

as *slow-reacting substance of anaphylaxis (SRS-A).* These have potent smooth muscle-stimulating activity and probably play a significant pathophysiologic role in immediate hypersensitivity and inflammatory reactions in certain individuals. The first group of enzymes mentioned are referred to collectively as *prostaglandin synthetase* and are inhibited by a number of pharmaceuticals, including aspirin, indomethacin, ibuprofen, flurbiprofen, and certain other nonsteroidal antiinflammatory agents. They seem to work predominantly on the cyclooxygenase aspect of that enzyme complex. The shunting of substrate degradation from the prostaglandin system to the leukotriene system may be responsible for the allergic response to aspirin seen in patients with asthma, nasal polyps, and aspirin hypersensitivity (Sampter's triad). PGE2 appears to act by increasing the concentration of cAMP in peripheral blood macrophages. This change in cAMP parallels its inhibitory effect on mitogenesis.

Prostaglandins that do not inhibit mitogenesis fail to stimulate cAMP increases. The platelet aggregation inhibited by PGE appears to be independent of cAMP. Cell surface receptors for prostaglandins have been identified. Cell surface receptors involved in the responses that increase the cAMP of course, must be coupled with the enzyme adenylate cyclase. There are additional uptake mechanisms for prostaglandins; therefore, effects of the prostaglandins on immune function may be independent of the adenylate cyclase stimulation.

Care should be taken when evaluating reports about the effects of the prostaglandins on the immune system, as much of the research is conducted with prostaglandin synthesis inhibitors, such as indomethacin. In high concentrations they may have a toxic effect on lymphocytes and, in addition, affect other enzyme pathways. A minimum of two structurally unrelated prostaglandin synthesis inhibitors should be offered as evidence of prostaglandin activity; in addition, the exogenous supply of the specific prostaglandin involved should return the system to a pre-PG-synthetase inhibitor state.

With regard to tumor immunology, mice injected with B-16 melanoma tumor cells have been shown to have decreased antibody response after intraperitoneal injections of antigen (sheep red blood cells). Indomethacin reverses this response. Indomethacin treatment has been shown to enhance the immunotherapeutic effect of *Corynebacterium parvum* or BCG injections in tumorous mice. Prostaglandins can directly inhibit the growth of some tumor cells in culture, and indomethacin and other prostaglandin synthesis inhibitors can enhance tumor growth. Whether endogenous prostaglandin production enhances or inhibits tumor growth is unclear. Panje (1981), however, has pointed out that at least some patients with squamous cell carcinoma of the head and neck appear to respond quite favorably to indomethacin.

Using indomethacin, Blitzer and Huang (1983) were able to dramatically suppress epidermoid carcinomas in rats. Indomethacin almost totally reverses the depressed phytohemagglutinin response in lymphocytes in Hodgkin's disease. This phenomenon appears to be significant, as mononuclear cells from patients with Hodgkin's disease appear to produce fourfold more PGE2 than normal. Prostaglandin suppression, however, does not appear to be involved in the antigen processing observed with disseminated malignancy of other types. The anergy that is seen in advancing age, however, may be related, in part, to prostaglandins, as peripheral blood macrophages from healthy subjects over 70 years of age are much more sensitive to the inhibition by PGE2 than are those of younger subjects. This must be interpreted in the context of a decrease in the antigen-responsive cells in this age group and

a longer cell division process then the cells are responsive (Goodwin and Webb, 1980).

Whether prostaglandins have a significant role in the control of tumor cell growth (as opposed to immunosuppression), irrespective of their effect on the lymphocyte multiplication, is debatable. Prostaglandins appear to participate in a negative-feedback production of CSF derived from monocytes. Tumors of the hemopoietic system undergo differentiation in the presence of prostaglandins in vitro. Because prostaglandins stimulate bone resorption in vitro, an effort has been made to treat hypercalcemic tumor patients with indomethacin, but it generally has been disappointing.

One area of interest is the role that prostaglandins may have on the metastatic behavior of malignancies. Evidence from experiments with lung and melanoma tumors in mice suggests that prostaglandin prostacycline produced from the same arachidonic acid precursor inhibits metastasis, and a prostacycline-enhancing compound appears to have antimetastatic effects in vivo. The response may be the natural counterpart for the experiments observed with prostaglandin synthesis inhibitors.

Prostaglandins are not the only immunosuppressive factors that tumors produce or induce. Roth et al (1983) have reported suppression of lymphocyte growth with extracts of human lung carcinomas, melanomas, and sarcomas. We have observed reversal of the skin test anergy associated with head and neck squamous carcinoma during cimetidine therapy in seven of nine untreated patients. The mechanism for histamine type 2 receptor suppression of lymphocytes is mentioned elsewhere in this chapter. Immune complexes and other substances that are immunosuppressive are sure to be identified as research continues into the abnormal responses that patients have to their tumors.

Tumor immunotherapy

Methods to provide immunotherapy for patients with cancer can be classified as active or passive from the patient's perspective and as specific or generalized from an immunologic perspective (Ferguson and Schmidtke, 1979). Once the patient has a tumor, very little current, active, specific therapy can be initiated. Attempts to generate vaccines for one's own tumor have been tried, but with little success. As identified previously, antibodies to tumors can be found in a variety of patients, but their value is unknown.

The revolution of tumor virology and viral genetics has allowed application of this technology to the production of certain viral vaccines that can be produced in either mammalian or bacterial cells. It is possible to produce vaccines for viruses that have oncologic potential, such as EBV and herpesvirus. In the future they may provide populations of individuals with a much different predisposition for developing head and neck cancer, even if placed in physical circumstances where they encounter the carcinogens that we usually associate with increases in head and neck cancer (that is, tobacco and ethanol).

Theories have been proposed about how an individual cancer patient's bone marrow or peripheral blood lymphocytes could be removed, stimulated to react with their tumors, and then reinjected into the patient so that humoral and cell-mediated immunologic activities could be enhanced (Patterson et al, 1982). It seems more likely that pharmacologic agents that allow this enhancement to take place in vitro, such as prostaglandin inhibitors and H_2 histamine

inhibitors, may allow favorable immunologic activities to take place.

Reports of increased survival of patients with lung cancer in a subgroup noted to have postsurgical empyema sparked a great deal of interest in nonspecific augmentation of the immune system. However, several problems were generated by the initial report. The first is that the numbers in the initial report were quite small: 18 patients in the empyema group and 34 in the control group. Attempts to reproduce this data using other nonspecific immunologic stimulants have been unsuccessful. Stimulants have included *C. parvum,* BCG, *Bordetella pertussis*, *Pseudomonas,* and synthetic agents, including glucon and polyribonucleotides. Theoretic agents that can improve or restore T-cell functions in addition to BCG include inoculation with *B. pertussis* and treatment with levamisole or viomycin. Similar agents indicated previously are also thought to increase B-cell functions. Because endotoxin induces interferon production, these agents could have a positive beneficial result by that route as well.

Recently a greater interest has been observed in the use of exogenous treatment of head and neck tumors with interferon as a single agent. The most dramatic positive effect seen in interferon treatment trials has been in the treatment of laryngeal papilloma. In uncontrolled trials, approximately 85% of the patients treated have either been stabilized or their diseases have improved. A small remaining percentage appears to be refractory to the effects of interferons. Although central nervous system/behavioural changes appear to be limiting factor in treatment with interferon, leukopenia and liver toxicity are also significant side effects. A persistent fever is seen in a few patients, although fever accompanied by tachyphylaxis is common in the treatment of many patients. Local irritation at the injection site has also been reported. Cardiac ectopy resulting in death has been reported in a few patients, but appears to occur exclusively in patients who had previous serious cardiac illness or had received cardiotoxic drugs.

Other head and neck diseases in which interferon has been reported to have beneficial effects include non-Hodgkin's lymphomas and, more recently, malignant melanomas. The action of interferon may be enhanced by cimetidine in the treatment of melanomas, the theory being that interferon reduces the helper/inducer T-cell activity to suppressor/cytotoxic T-cell activity and that cimetidine can reverse this activity, as there is an H_2 histamine receptor on suppressor T cells.

The malignant gammopathies, including malignant melanomas, occasionally present in the head and neck region and have also been shown to be sensitive to the interferons. There is a single case report of a nasopharyngeal carcinoma refractory to other forms of therapy that responded quite dramatically to interferon, but this single report has not been followed up. A group of Yugoslavian investigators have used interferon in a variety of head and neck tumors, including squamous cell carcinomas and basal cell carcinomas with results that they report as being favorable. However, their criteria do not correspond to those usually used for evaluation of chemotherapeutic agents (Richtsmeier, 1983).

Direct treatment of patients with IL-2, as well as other leukokines or cytokines, may be beneficial (Cortesina et al, 1988, Rivoltini et al, 1990, Sacchi et al, 1990, Schantz et al, 1990). One of the problems with patients with head and neck cancer is their debilitated nutritional state; such activity adversely affects the immunologic system and may contribute

to the downward spiral observed in many patients with advanced tumors. Without adequate nutritional support, other forms of immunotherapy probably have reduced benefit.

The concept of adoptive immunotherapy of cancer patients was extended to more than cytokine administration by Rosenberg and his group at the Surgery Branch of the NIH through the treatment of cancers with autologous lymphocytes, expanded in vitro with IL-3 (Rosenberg et al, 1985, 1987). In addition to the growth of the cells under the influence of IL-2, an increase in the tumor killing potential of these "lymphokine activated killer lymphocytes" (LAKs) was observed. The LAK cells would recognize fresh tumor cells and kill them with an increased efficiency: IL-2 circumvents the need for T_4 (helper) lymphocytes in the production of antitumor cytotoxic lymphocytes (Fearon, 1990). The original therapeutic plan was to use this adoptive immunotherapy for sarcomas, which have few therapeutic options other than surgery. The model developed in mice showed a dramatic effect against murine sarcomas, but unfortunately this has not been observed in humans. However, results in melanoma and renal cell carcinoma are promising. The initial protocols showed that the LAK cells (expanded in vitro from peripheral blood) need concurrent IL-2 infusion for effect. The early results soon led to the observation that tumor-specific killer lymphocytes could be expanded from the lymphocytes that infiltrated the other tumors (TILs) and IL-2 plus TILs are now being used in SCC and other cancers. Results with IL-2 alone as a systemic agent have been disappointing, in part due to the toxicity of IL-2. Il-2 induces TNF-alpha and each has been shown to produce an extensive capillary leak resulting in massive pulmonary edema if not carefully monitored and treated. Regional IL-2 therapy does not cause the toxic symptoms and has great promise for head and SCC, which has a large local control component.

Immunodeficiency

The lack of one or more of the types of immunologic responses, either blood- or tissue-mediated immunity, can give rise to a clinical syndrome in which infectious diseases or tumors (or both) may arise. There are many different types of immunodeficiencies. The mode of inheritance of many congenital immunodeficiencies has been worked out and reviewed (Rosen et al, 1984). Recently attention has been focused on acquired immunodeficiency states.

Several antibody-deficient states are of interest to surgeons. The most obvious condition is that of agammaglobulinemia, an X-linked inherited disorder, which becomes evident in patients after the first 6 to 9 months of life. After that time, the maternally transmitted immunoglobulin (IgG) has been metabolized, and patients have repeated infections with extracellular pyogenic organisms. Because T-cell function is intact, intracellular parasites, such as viral infections, are not a problem. A notable exception is a persistent infection with enteroviruses of the poliovirus group. This syndrome can be diagnosed in patients by obtaining quantitative serum immunoglobulin levels and noting that IgG, IgA, and IgM levels are all below 2 normal standard deviations from the mean, which is usually less than 100 mg/dL of total immunoglobulin.

Because immunoglobulins are made by B cells, the EBV receptor-bearing lymphocytes are not present, and EBV is not a major problem. patients are treated with injections of human pooled immunoglobulin; an initial dose of approximately 200 mg/kg is required, with

a repeat maintenance dose of 100 mg/kg every 3 or 4 weeks. The dose is arbitrary. There is no means to provide secretory IgA antibody to the mucous membranes and surfaces. This fact must be kept in mind when diagnosing and treating patients with head and neck diseases.

Selective IgA deficiency is the most common symptom for the physician to encounter in immunodeficient patients. Infections occurring in the respiratory, gastrointestinal, and urogenital tracts in patients occur with selective IgA deficiency. Interestingly, however, the disease has been reported in apparently healthy individuals. The same types of infections that are a problem for patients with agammaglobulinema are seen in this disease as well. It is of some interest that IgM antibodies, which are synthesized in the tissues that secrete fluids, can combine with the secretory subunit for the local secretion of antibody in a manner similar to that reported for IgA. One of the protective roles of IgA may be in preventing absorption of large antigens from the gastrointestinal tract. This role is inferred because of the increased incidence of allergy to dairy products seen in patients with secretory IgA deficiency.

An induced selective IgA deficiency has been reported in patients taking phenytoin sodium and penicillamine that may be reversed when the drug is stopped. One of the more interesting aspects of this disease is the identification of antibody to IgA in the sera of a large percentage (40%) of patients with selective IgA deficiency. (See the discussion of idiotypes in the section on autoimmune diseases.) These antibodies can fix complement, and near-fatal anaphylactic reactions have occurred in patients with IgA deficiency who received intravenous administration of blood products. Because of this problem, only repeatedly washed erythrocytes or blood products from other IgA-deficient persons can be administered safely. Since the patient's blood contains antibodies to IgA, the intravenous administration of blood produces can result in antigen-antibody complex formation, complement fixation, histamine release, and serious illness. At best, the administered IgA will be inactivated by the patient's own serum. Because of this phenomenon, there is no recommended treatment for selective IgA deficiency beyond the vigorous appropriate antimicrobial therapy for identified infections.

T-cell immunodeficiency disorders

The most obvious immunodeficiency of T cells comes from aplasia or hypoplasia of the thymus, which is seen in the syndrome of thymic hypoplasia or DiGeorge syndrome, which is associated with hypoplasia of the parathyroid glands (Rosen et al, 1984). The clinical disease of DiGeorge syndrome is identified often by hypocalcemic tetany during the neonatal period. This tetany comes from abnormalities in the third and fourth pharyngeal pouches during embryogenesis; and other structures in this areas may also show abnormalities that include the great vessels, esophagus, uvula, mandible, and auricles. Congenital heart disease may be associated. The spectrum of severity of disease is seen because of the variable degree of hypoplasia that is usually seen rather than total aplasia of the thymus and parathyroid glands. As would be expected, serum immunoglobulins are usually found in normal concentrations, although higher than normal levels of IgE may be seen, as well as decreased levels of IgA. This inherited disorder gives rise to a syndrome that can be identified in older individuals as having recurrent infections with opportunistic agents, wasting-like syndrome with weight loss and fever often accompanied by diarrhea, potentially fatal dissemination after vaccination with live virus such as vaccinia, and an unusually high incidence of malignancy. Given the opportunity, the patients may display a graft-versus-host reaction if given unmatched allogeneic bone marrow cells or blood products, and they have delayed cutaneous

skin test response.

Because problems are seen with both selective immunoglobulin and T-cell immunodeficiencies, it is not surprising that combined immunodeficiency disorders have been identified. Indeed, a congenital phenomenon occurs wherein patients appear to have no ability to distinguish self from foreign organisms. This is the severe immunologic reconstitution with bone marrow transplants, enzyme replacement, or gontobiotic isolation, because the patients usually die in the first year of life if treatment is not instituted properly. There are several major categories of this disorder. An autosomal recessive, severe combined immunodeficiency disease is identified, as well as an X-length recessive form; and a severe combined immunodeficiency with leukopenia, referred to as *reticular dysgenesis,* is observed.

In some patients with severe combined immunodeficiency disorders, the interrelationship between T and B cells can be observed in that they have normal numbers of B lymphocytes, which do not differentiate further in the absence of T-cell activity, as the T-cell portion of the deficiency is profound. These patients have a nearly total lack of cellular immunofunction that is demonstrated by a variety of in vitro tests. They have delayed cutaneous anergy in vivo and an inability to reject transplants. Their serum immunoglobulin concentrations are usually extremely low, and after immunization no specific antibody production can be detected. They highlight the controlling role T cells play in B-cell maturation.

Related to the defect of cellular immunology is the phenomenon of the disorder of phagocytic cell function. Because phagocytosis involves chemotaxis, opsinogenation, ingestion, and intracellular killing, defects may be observed during one or more of these phases. The physician usually observes these defects, if at all, in patients with chronic granulomatous disease, which is usually seen in childhood. In these patients neutrophils are defective in their ability to kill catalase-positive bacteria, such as *Staphylococcus aureus,* even though they normally phagocytize them and have intact antibody production. The patients may have adenopathy with or without drainage, pneumonia, hepatomegaly with abscesses, osteomyelitis, splenomegaly, and dermatitis, usually beginning before 1 year of age. It is unusual, fatal disorder wherein intracellular bacteria, such as the catalase-positive staphylococci, can grow and kill the cells that have ingested them. Catalase-negative organisms, such as pneumococci and streptococci, can be killed effectively. Patients theoretically should benefit most with treatment using antibiotics that have improved intracellular penetration, such as clindamycin or erythromycin.

The physician who deals with head and neck cancer is all too aware of the deleterious effects of malnutrition, and in the area of immunology these have been clearly documented in the literature. Protein malnutrition in particular may be manifested by thymic atrophy and a decrease in peripheral T-cell activity, as well as a decrease in circulating immunoglobulins.

There is no question that pregnancy confers with it a certain amount of immunologic tolerance. The pregnant uterus does not reject the autografted placenta, and T-cell responses to stimulation appear depressed. In addition, certain hormonelike factors may participate, as low levels of circulating interferons have been identified in cord blood found in all specimens examined, and these factors may contribute to the immunologic tolerance state (Richtsmeier, 1984a). The effects of aging on the immune system appear to result more from a gradual

depletion of immunocytes and stem cell precursors than from specific responses that can be carried out. Howard (1979) has reviewed the immunosuppressive effect of surgery and general anesthesia. Decreases in the number of immunologically active cells in peripheral blood as well as in their functions have been observed. Phagocytosis is also transiently affected. In this situation it is again difficult to separate the effects of the operation or anesthetic from those of the disease that mandated the surgical intervention.

AIDS

The work on inherited immunodeficiencies has been overshadowed by the enormous effort spent on working with an acquired immunodeficiency which began to appear in the USA in 1979 now known to be associated with type IV immunodeficiency virus. The new disease is seen now as acquired immune deficiency states demonstrated by the patient having an infection that is indicative of underlying cellular immunodeficiency such as infection with *Pneumocystis carinii* or development of Kaposi's sarcoma. The immunodeficiencies are not associated with other diseases that are known to cause immunosuppression, such as EBV infections (Carney et al, 1981), or with secondary causes such as malnutrition associated with head and neck cancer. Patients have been identified in both the adult and pediatric populations (Scott et al, 1984). The disease has been associated with sexual activity involving multiple partners, shared intravenous needles, and blood transfusions (Curran et al, 1984). Diagnosis of this disease rests largely on clinical grounds but has strong laboratory support. Patients have reversed T-cell ratios. The normal helper (inducer)/suppressor cytotoxic T-cell ratio is 2:1, in AIDS patients this ratio is usually less than 1:1 and symptoms of the immunodeficiency appear when the absolute T4 lymphocyte count falls below 200 cells/dL.

This disease has received much attention in the medical literature for several reasons. One is the public hysteria that accompanied its identification and the fear that the disease would spread to the heterosexual population; another is the scientific interest in this disease. The disease itself appears to have epidemiologic findings that clearly indicate an infectious etiologic agent, which is a variant of the T-cell leukemia virus (HTLV-III) (Groopman et al, 1984). The virus is not particularly infectious, and repeated contact or direct injection of infected blood is usually necessary. Infection of patients with blood that did not contain virus particles but only infected T4 lymphocytes has raised the concept of "Trojan horse" leukocytes transmitting the disease (Shearer, 1983).

The virus receptor attaches to the T4 Ag on the surface of the lymphocyte - the same one recognized by Abs to the CD4 locus. The specificity for an infection of T-helper cells is therefore obvious. Small amounts of T4 Ag are expressed on other cells (including some in the central nervous system and antigen-present, accessory cells) so that initiation of the infection can begin and persist in other cells of the body. The main cell infected is the T4 lymphocyte. Initially there is no problem because the virus is a retrovirus and inserts its complementary DNA (cDNA) into the cell's genetic material. The problem begins when the virus (or the patient's body or a treatment) begins to kill the infected T4 lymphocytes. The cell loss is irreplaceable because the T4 lymphocyte is central to the primary lymphocyte response. The infected, T4 lymphocyte-depleted patient manifests an inability to handle low virulence infectious or low-grade malignancies, which never present a problem for an organism that has functioning T4 lymphocytes.

It has been difficult to prepare a vaccine against the AIDS virus because of its binding to the locus specific for the helper T cell and the complementary binding site identification locus on other cells of the immune system.

The incidence of Kaposi's sarcoma in AIDS, which had previously been identified as a rare indolent form of cancer present in patients of Eastern European origin, is unknown. The disease has been likened to an "oncologic looking glass" by Groopman and Gottlieb (1982) and has intrigued investigators because of its epidemiologic public health and social aspects in addition to the oncologic, virologic, and immunologic aspects just mentioned. Diseases seen with AIDS in addition to *P. carinii* are protozoa, such as *Cryptosporidium* and *Isospora,* toxoplasmosis, atypical mycobacteria, *Legionella, Candida,* and *Cryptococcus* disseminated in infections, as well as disseminated herpes simplex, varicella zooster, and adenovirus.

Evaluating a patient for the possibility of having an immunodeficiency begins with noting a failure of the individual to respond normally to therapy for a known infection or noting disseminated infection with an unusual organism. Generally, both humoral and cell-mediated systems require investigation. Quantitative immunoglobulin electrophoresis can identify many problems in this regard; however, most patients with selective deficiencies have normal immunoglobulin levels. Traditional hypersensitivity skin testing is a simple, tried-and-true method of detecting cell-mediated immune deficiencies. Observing the normal reaction of induration and erythema, which appears 48 hours after injection of antigen, is strong evidence that a patient is unlikely to have a serious defect in cell-mediated immunity.

Many in vivo tests can be carried out on the patient's leukocytes. The first step is to evaluate their morphology and quantitate them. Next, their function can be measured with appropriate activity noted, such as phagocytosis, intracellular killing, and diapedesis for granulocytes; blastogenesis, lymphokine production, and immunoglobulin production for various lymphocytes; and quantitation of subsets of lymphocytes.

Clinical evaluation of a patient suspected of having AIDS usually begins with identification of the patient's chronic fatigue, weight loss, fever, and lymphadenopathy. The patient may have skin lesions associated with Kaposi's sarcoma. In patients evaluated initially for head and neck disease, 44% had such dermal lesions, 39% had oropharyngeal lesions, and 32% had cervical adenopathy. One third of the patients studied died of opportunistic infections. The head and neck lesions often herald more extensive disease. In addition, patients may have a persistent cough or diarrhea; or they may have easy bleeding, indicating problems with the hemopoietic system, and neurologic and psychiatric findings, which suggest infection with cytomegalovirus, multifocal leukoencephalopathy, or toxoplasmosis. Other symptoms may suggest cryptococcal bacterial meningitis. Ophthalmic signs include papilledema, uveitis, retinal hemorrhage, vascular occlusion, and cottonwood exudates.

Because information about AIDS is developing so rapidly, it is certain that much more will be known about this disease soon after this text is published. It is important to keep in mind the process by which these discoveries have been made, which has involved a relatively straightforward examination of the immune system and its rapid application to clinical medicine. Unquestionably, these patients slide along the lines of the immunologic tetrahedron that relates infectious diseases to oncology and immune deficiencies to the evolution of cancers. Also without question, the study of this disease will add enormously to the

understanding of other malignancies, unexplained lymphadenopathy, and transient immunologic defects that occur in a variety of patient populations in association with a variety of infectious agents.

Autoimmune Diseases

As discussed in previous sections, particularly those related to humoral antibody production and cell-mediated immunity, the cornerstone of the normal function of these processes is the correct identification of a foreign substance by the host so that the host can respond to it in a way that renders the foreign substance harmless to the organism as a whole. The undesirable aspects of IgE-mediated immune complex disease are all too clear to anyone who sees patients with allergic problems, but the distinction between IgE-mediated disease and IgE-mediated health is not always clear. On the other hand, immune complexes that form in the body can cause a variety of problems.

As discussed in the section on infectious diseases, relative concentrations of antibody and antigen are particularly important in determining the type of precipitation pattern that develops under a circumstance wherein the relative amounts of antigen and antibody do not form precipitants large enough to be phagocytized. If the antigen itself is not particulate, these small complexes can be distributed throughout the body and cause clinically recognizable diseases where they are deposited. In this way a normal antigen-antibody response can move the site of inflammation from the original site of entry to another site. They can cause vasculitis, glomerular nephritis, and arthritis, depending on the organ in which they are deposited. The larger complexes, in which more than one immunoglobulin molecule exists per antigen molecule, are more often deposited in the subendothelial and mesangial areas of the kidney. The inflammation that develops after collection of such deposition can produce clinical symptoms such as arthritis, particularly after activation of the complement system, basal active peptides, and chemotactic factors.

Once the generalized inflammatory response has been initiated, T-cells, thirteen cells, and mononuclear cells arrive at the site, and the maturation process continues. The model of heterologous serum that causes experimental acute serum sickness should be reviewed (Gilliand and Mannik, 1983). However, current practices do not include the use of antigenic foreign substances such as heterologous serum. Today immune complex diseases are more commonly found in association with drugs and foreign proteins used as vaccines. Antilymphocyte antibody (Lawley et al, 1984) and monoclonal antibody treatments may make the syndrome of serum sickness more familiar (Gilliand, 1984). Because the antigen-antibody complex necessarily involves at least antibody made by the individual patient, immunofluorescent study of biopsy materials aimed at identifying the precipitation of antibodies in the patient's tissues can often help elucidate the nature of the disorder.

Although antigen-antibody complex deposition is a common serious form of autoimmune disease, it is not as dramatic as the diseases that occur as a result of the action of antibody that directly combines with either cell surfaces or intracellular structures, or with sensitized T lymphocytes that are directed against host tissues.

A common example of the first type mentioned is Hashimoto's thyroiditis. Graves' disease, wherein hyperfunction of the gland is caused by thyroid-stimulating immunoglobulins

(TSI) reacting with the receptor for thyroid-stimulating hormone (TSH), is actually a fifth type of immunologic disease. This disease is different from thyroiditis because once the antigen-antibody (TSH receptor-TSI) complex forms, the same adenylate cyclase mechanism initiates increased secretion of thyroid hormones just as if TSH had come in contact with the cell. No inflammatory process takes place.

An example of the cell-mediated type of disease is more difficult to find and probably does not exist in a pure form. This may be the basis for some complex phenomena such as acute sensorineural hearing loss (Veldman et al, 1984a). Deposition of antigen-antibody complexes in different tissues initiates the complement system and T-cell activation in the same manner as other antigens. In addition, activation of the NK system may be a major component of processes seen in disorders such as Sjögren's syndrome, wherein lymphocytic infiltrates are a primary feature.

Given the great number of possible antigens, the extent of the immune response potential of a given organism is impressive. Two major theories have been postulated to explain the ability of the lymphocytes to form antigen-combining sites in the variable region of the immunoglobulin molecule (Stites et al, 1982). The first is the somatic mutation theory, which dictates that the cell "makes up" the variable portion of the immunoglobulin molecule to conform to some template that is perceived (presumably) by the macrophage group. The second theory is one of a germline concept, which postulates that the cell's genetic material already contains genes coated for immunoglobulin molecules that could be made against any antigen encountered. Using recombinant DNA technology, genetic investigations of immunoglobulin genes indicate that probably both of these concepts contribute to the ability of a cell to make a distinct antibody molecule for a given antigen. Because there are only enough genes to code for 104 to 105 possibilities, this number must be enlarged by a process of genetic recombination, allowing a diverse selection of variable portions of antibody molecules to be greater than a million. At what stage of differentiation the cell is able to set up such a genetic rearrangement or selection is not clear; however, once this clone has been established, memory is kept intact. On the other hand, most B cells live only a few days; thus the stem cells must maintain this diversity of genetic material. In that sense, the organism is continually resampling and reestablishing what is self and what is foreign.

What happens to the T-cell antigen-combining system is much less clear. The nature of T-cell receptors for antigens, their affinity-binding coefficients, T-cell clonal selection, and other aspects of this response are still under intensive investigation. It is clear that MHC antigens are an important part of the T-cell receptor library. Cells that have matured in the thymus are able to identify self-MHC antigens. This is in itself a form of autoimmunity. It appears that T cells must recognize both self- and non-self-antigens to trigger T-cell differentiation. Certainly this phenomenon of T-cell evaluation of "self" antigens is extremely important in the homeostatic mechanisms of the organism. The need to identify both self- and non-self-antigens may be a mechanism by which T cells remain tissue directed. This need to find self, referred to as *MHC restriction phenomenon,* may change the cell's "interest" in binding the non-self-antigen. If one imagines the T lymphocyte placed in focus of a viral infection, the T lymphocyte will be exposed to both a free virus and virus-infected cells that have some viral antigens expressed on their surfaces, as well as normal MHC antigens. The T lymphocyte will then be directed to address the source of the problem (that is, the virus-infected cell as opposed to free virions) because it must see both antigens to act appropriately.

One can easily imagine how important this function would be in chronic viral infections such as with cytomegalovirus or herpesvirus, as well as different surface antigens that may appear during viral oncolytic transformation.

Idiotypes

A discussion on self or non-self would not be complete without at least a brief treatment of the concept of idiotypes. Since serum sickness is a disease resulting from human antibody combining with equine sera, it is obvious that the antibody molecules of one organism may have antigenic determinants that can be reacted on by another. Immunologists have been interested particularly in the variable portion of the immunoglobulin and have referred to determinants on portions of immunoglobulin molecules such as idiotypes. The antibodies produced against these areas are termed *antiidiotypes* (Stites et al, 1982). The antiidiotype antibody then reacts only with a specific determinant on the variable portion of an immunoglobulin molecule. Antiidiotypes may come from any of the classes of antibodies discussed previously. What is important about this concept is that both the B and T cells express idiotypic determinants on their surfaces.

The exact nature of the T cell idiotype is not clear; however, the T-cell receptor can be thought of functionally as containing the variable portion of an immunoglobulin molecule. The number of cells that have different idiotypes expressed on their surfaces apparently is as large as the entire range of antibody specificities of the individual. The theory of immunologic control using idiotypes expressed on the surface of lymphocytes has been proposed by Jerne and expanded by others (Stites et al, 1984). According to this theory, after exposure to an antigen, cells make an antibody, which by the nature of its conformation and binding to the antigen, is indeed an idiotype. The production of this antibody-idiotype stimulates the synthesis of another antibody, which is complementary, fitting opposite the idiotype, and therefore directed against the antibody-combining site of a molecule. The structure of the confining site of this antiidiotype is referred to as an *idiotope* and is stereochemically similar to the original antigen that began this discussion.

The antiidiotypic clones that contain the idiotype may be of the T- or B-cell series and, within the T-cell series, may be helper or suppressor in function, giving these cells the ability to provide a positive or negative feedback in the immune response. One can imagine the young organism in a steady state that is disturbed by the introduction of antigens freeing cells from their helper or suppressor states or influencing the organism toward a new steady state. This new steady state could be characterized by either immune memory or tolerance, depending on the helper-suppressor nature of the interaction. When experimentally produced anti-idiotypic antibodies were administered to test animals, suppression or enhancement of the complementary idiotypic antibody was produced. This appeared to occur through the reaction of different sets of B cells and helper or suppressor T cells found in the antiidiotypic network. More detailed explanations of this system can be found elsewhere (Stites et al, 1982).

The importance of this mechanism is twofold. First, it provides an explanation for the regulation of production of antibodies, B cells, and T cells; second, it provides a mechanism of immunologic control that may be able to be manipulated to a given patient's advantage once more is known about this system. Preliminary reports have indicated that passive administration of antiidiotypic antibody to a patient with B-cell lymphoma has led to dramatic

response (Miller et al, 1982).

Explanations of how cells become tolerant to new cells of the same organism have given rise to a concept of deletion of certain clones of antibody-producing cells, which largely takes place during embryogenesis. As B cells mature, their ability to delete certain clones decreases significantly. However, even adult animals can become tolerant to antigens if the immature B cells are used. This function appears to continue throughout life as an active process. Complex molecules appear to be noted in reducing tolerance more than small simple ones. This interactivation appears to occur at the cell membrane level during the early life of B cells, resulting in inactivation of such cells and the functional loss of a clone that could be produced from it.

Another theory of induced tolerance suggests that large amounts of antigens are exposed to cells and inactivate a population of immunocompetent lymphocytes as the result of the antigen-antibody binding in an unfavorable environment. Whether the helper/suppressor T-cell system is involved or whether these mechanisms actually are used in the evolution of tolerance to certain antigens, such as those associated with tumors, is not clear. The macrophage is probably involved to some extent, although again the mechanism is unknown.

The hereditary disposition of an individual to develop an autoimmune disease and hormonal influences certainly must be important, as clinical diseases often fall in patterns characterized by particular MHC blood groups with strong sexual predominance.

When one examines the mechanism by which foreign substances are identified, it is easy to imagine how a drug attached to an individual's proteins or virus protein within a cell can trick the immune system into recognizing a portion of the patient's self as being foreign. Because many viruses use host phospholipids and mucoproteins to help stabilize the membranes during assembly of maturing viruses, it is not difficult to imagine that some of them could mistakenly be recognized as foreign. In addition, during the lysis of cells during viral infections, fragments of host cell DNA may be mistaken for viral DNA, particularly when one considers that host DNA is seldom exposed to the circulation outside of normal erythropoiesis. Although the mechanism by which autoimmune diseases occurs is poorly understood, even with the most common diseases such as systemic lupus erythematosus (SLE), it is not difficult to imagine the processes by which they could arise, but it is more difficult to prove such a mechanism. The opposite response of not recognizing foreign substances when they are present, of course, gives rise to the clinical situations identified with immunodeficiency syndromes and cancer. It should be remembered that antigens may appear at different times during an individual's life. They may have been present during embryogenesis, then genetically sequestrated during adult life, and, if later exposed, may initiate an immune response as if they were foreign antigens.

Because the exact mechanisms of specific autoimmune diseases are not known, a lengthy explanation of the processes involved cannot be provided.

Some additional findings about the immune system bear discussion. The hypothesis that SLE comes from a viral infection is supported by several observations. The first is from a model for SLE that can be developed in mice infected with a retrovirus. Antibodies to this retrovirus glycoprotein from immune complexes are observed in such patients. A viruslike

tubeoreticular substance found by electron microscopy and antigen interferon-antibody complexes have been found in these tubeoreticular substances in humans (Campbell et al, 1983).

Suppression of cell-mediated immunity is apparent in many patients with SLE, and there appears to be a genetic predisposition because of the high concordance of clinical SLE in monozygotic twins. Because lymphocytotoxic antibodies have been identified in household contacts with patients with SLE, the agent that causes it probably is not unique to this disease. Finally, a unique interferon has been identified in the sera of patients with a number of autoimmune diseases, SLE being the most common and giving rise to the highest titers. Whether interferon is a result of a viral infection or part of the disease process itself is not clear. Because interferon has so many effects on the immune system, it may well serve to manipulate this immunologic disease. This discussion of the basic science of autoimmune diseases cannot hope to encompass the clinical manifestations of these diseases. To help guide the reader in other areas of the text, a list of autoimmune diseases of the head and neck is provided in Table 14-4.

Table 14-4. Head and neck autoimmune diseases

Clinical disease	**Area of involvement**
Rheumatoid arthritis	Temporomandibular joint
	Larynx (cricoarytenoid joint, vocal cords, laryngeal musculature, laryngeal nerve)
	Middle ear ossicles
	Cervical spine
	Urethra and/or cervix
	Bowel
	Conjunctiva mucosa
Sjögren's syndrome	Major and minor salivary glands
	Lacrimal gland
	Mucous and sweat glands
SLE	Skin
	Oral mucosa
	Salivary glands
	Cranial nerves
	Cervical lymph nodes
Scleroderma	Connective tissue including skin and appendages
	Salivary glands
	Esophagus
	Polymyositis, dermatomyositis

Skin
Esophagus
Mouth
Parotid and tonsillar neoplasms

Pemphigus
Epidermoid basement membrane and intracellular antigens

Relapsing polychondritis
Ear, nose, respiratory tract
Eye
Great vessels
Joints

Vasculitis
See specific diseases for areas of involvement: hypersensitivity vasculitis, polyarteritis nodosa, Wegener's granulomatosis, Behçet's disease, Cogan's syndrome, polyarteritis, temporal arteritis

Thyroiditis
TSH receptors (Graves' disease)
Microsomes and thyroglobulin in Hashimoto's thyroiditis

Myasthenia gravis
Acetylcholine receptors

Rhinitis (allergic and vasomotor)
B_2-adrenergic receptor in mucosa.