
T H I R D E D I T I O N

PART A

GARNER AND KLINTWORTH'S

PATHOBIOLOGY
OF
OCULAR DISEASE

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Immunologic Processes in Disease

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INTRODUCTION

Many eye diseases represent the interaction of the immune system with ocular or periocular tissues. A good knowledge of the immune system and its interaction with the eye is necessary to understand the several ocular and ocular adnexal inflammatory diseases. This chapter outlines the general principles of immunology, discusses specific ocular manifestations of the immune response, and finally considers immunologic processes in ocular infections. A full discussion of immunology is beyond the scope of this text and many excellent reviews exist (1,21,531–3).

CELLS AND CELL SURFACE MARKERS OF THE IMMUNE SYSTEM

All the cells making up the immune system are derived from the hemopoietic stem cells of the bone marrow. Differentiation of the many cell types that make up the immune system has been greatly enhanced by the development of monoclonal and polyclonal antibodies that specifically react with surface glycoproteins on certain cell subtypes. These surface markers often help differentiate the status of a given cell as well. For example, many cells express certain surface molecules only when they are activated. These markers have been standardized and systematized by international workshops into a system of clusters of differentiation (CD) (Table 1). A group of glycoproteins called major histocompatibility complex (MHC) molecules—known as human leukocyte antigen (HLA) molecules in humans—have been identified as extremely important in predicting rejection following tissue transplantation. These surface molecules present antigens to immunocompetent cells, primarily T lymphocytes. Two classes of MHC molecules exist: class I molecules are found on all nucleated cells and present antigen derived from the intracellular components, such as tumor or viral

Table 1 List of Selected CD Antigens

CD antigen	Cellular expression	Functions	Synonyms
CD1	Cortical thymocytes, Langerhans cells, dendritic cells, B cells, intestinal epithelium, smooth muscle, blood vessels	MHC class I-like molecule with special role in presentation of lipid molecules	
CD2	T cells, thymocytes, NK cells	Adhesion molecule binding CD58 (LFA-3)	T11, LFA-2
CD3	Thymocytes, T cells	Associated with the T-cell antigen receptor (TCR). Required for surface expression and activation TCR	T3
CD4	Thymocyte subsets, Th1 cells, Th2 cells, monocytes, macrophages	Co-receptor of MHC class II molecule	T4, L3T4
CD8	Thymocyte subsets, cytotoxic T cells	Co-receptor of MHC class I molecule	T8, Lyt2,3
CD11a	Leukocytes	Subunit of integrin LFA-1	LFA-1
CD11b	Granulocytes, macrophages	Binds CD54, complement component iC3b, and extracellular matrix proteins	Mac-1
CD11c	Granulocytes, macrophages, lymphocytes		Adhesion molecule, binds fibrinogen
CD16	Neutrophils, NK cells, macrophages	Low-affinity Fc receptor, mediates phagocytosis and antibody-dependent cell-mediated cytotoxicity	Fc γ RIII
CD23	Mature B cells, activated macrophages, eosinophils, follicular dendritic cells, platelets	Low-affinity receptor for IgE	Fc ϵ RIII
CD28	T-cell subsets, activated B cells	Activation of naïve T cells, receptor for costimulatory signal. Binds CD80 (B7.1) and CD86 (B7.2)	
CD40	B cells, macrophages, dendritic cells, basal epithelial cells	Binds CD154 (CD40L); receptor for costimulatory signal for B cells; promotes growth, differentiation, and isotype switching of B cells	
CD45	All hematopoietic cells	Tyrosine phosphatase	Leukocyte common antigen

Note: For a complete list visit the Website of the human leukocyte differentiation antigens/human cell differentiation molecules at <http://www.hlda8.org> or protein reviews provided by the NIH at <http://www.ncbi.nlm.gov/prov/>.

antigens, to cytotoxic CD8⁺ T lymphocytes; class II molecules are found predominantly on professional antigen-presenting cells (APCs), such as dendritic cells, macrophages and B cells, and these cells present extracellular antigens that have been endocytosed, such as fungal or bacterial antigens, to helper CD4⁺ T lymphocytes.

Polymorphonuclear leukocytes (PMNs), eosinophilic leukocytes, mast cells, macrophages, dendritic cells, and microglia (4) are derived from the myeloid progenitor cell in the bone marrow. Among these, the cells containing intracytoplasmic granules are divided into PMNs, eosinophils and basophils based on differential staining of their granules. Most circulating granulocytes are PMNs. These cells express surface receptors for immunoglobulin (Ig)G and certain complement components. Following upregulation of adhesion molecules on vascular endothelial cells in an inflamed tissue, chemotactic agents such as complement components, fibrinolytic and kinin system components, and leukocyte, platelet or bacterial products cause circulating PMNs to migrate into the involved tissue and release inflammatory mediators into the surrounding tissue. Eosinophils possess surface receptors for IgE, IgG and complement

components. These cells are commonly found in allergic and parasitic conditions. Like PMNs eosinophils degranulate when activated. Mast cells reside near small blood vessels and they are distributed in the human conjunctiva, uvea, and other ocular tissues. These cells are numerous in the conjunctiva and 5000 to 6000 cells/mm² are present around the vascular elements (3). These cells are important activators of the allergic response and mucosal immunity. When antigen binds to two adjacent IgE molecules on the mast cell surface, the mast cell degranulates releasing vasoactive amines, such as histamine, which increase vascular permeability and allow immune complexes to deposit in the vessel wall. Basophils express surface receptors for IgE, IgG and complement components and their function appears to be similar to that of mast cells.

Dendritic cells and macrophages are typical APCs since they constitutively express MHC class II molecules, which present antigen to CD4⁺ T lymphocytes (Fig. 1). They also express IgG receptors which can be used to engulf extracellular antigens after they are coated with IgG, a reaction specifically known as opsonization. Moreover, APCs stimulate lymphocytes by cytokine production, such as

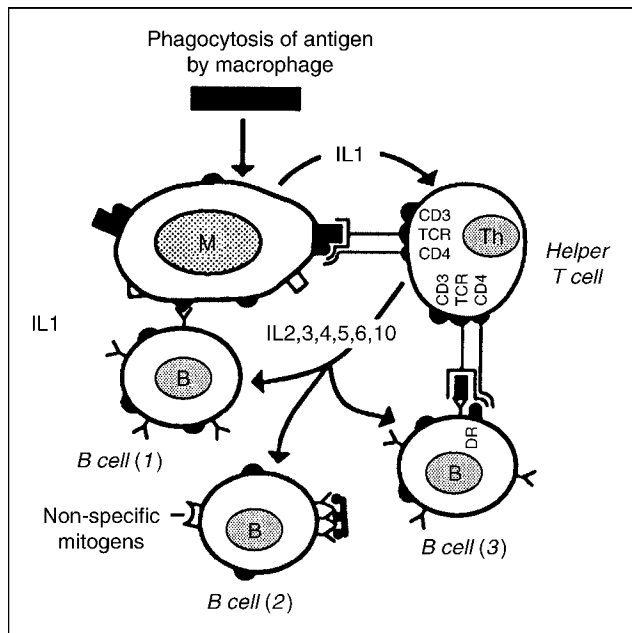


Figure 1 Network of cellular interactions in immune regulation. Antigen taken up by macrophages (M) is broken into fragments, linked with class II HLA, and presented to a helper T cell (Th). T-cell receptor (TCR) and the CD4 molecules recognize the modified antigen on the surface of the macrophage. In addition to CD3, which stabilizes the TCR, other accessory molecules on helper T cells bind to corresponding receptors on the macrophage. These interactions are a prerequisite for T-cell activation. Subsequent B-cell activation can develop in three ways: (i) antigen can be presented directly by a macrophage in the presence of T cell-derived IL3, IL4, IL5, IL6, and IL10; (ii) alternatively, antigen, particularly a polymer with repeating peptide units, even when it is unprocessed, can sometimes bind directly and promote B-cell activation; or (iii) B cells may also express class II HLA and therefore behave as antigen-presenting cells and stimulate TH to initiate an immune response. Source: Courtesy of Dr. Amjad H.S. Rahi.

interleukin (IL) 1 and tumor necrosis factor- α (TNF α), and by surface expression of costimulatory molecules required for successful antigen presentation (discussed in greater detail later in this section). These cells secrete a host of other immune mediators including proteases, collagenases, angiotensin-converting enzyme, lysozyme, IL6, and macrophage colony-stimulating factor and reactive oxygen and nitrogen species. Specialized macrophages exist in many tissues such as the Kupffer cells of the liver, dendritic histiocytes of lymphoid organs and uveal tissue, Langerhans cells of the skin, lymph nodes, conjunctiva and cornea, and microglia of the retina and central nervous system (CNS).

A common lymphoid progenitor gives rise to lymphocytes. Lymphocytes are 7 to 8 μ m, round mononuclear cells (Fig. 2). A subset of them, B cells, mature in the bone marrow and produce antibodies. They are the primary cells responsible for adaptive

humoral immunity. B lymphocytes can recognize extracellular antigens not processed or presented by other immune cells by means of surface antibody receptors and the antibody which the cell itself produces and secretes. Antibodies are divided into five classes by the characteristics of the constant regions of the antibody molecule. IgM is the first antibody class produced to a specific antigen. Typically during a second exposure to the same antigen, the B cell is activated either by the surrounding cytokine milieu or by some bacterial polysaccharides and lipopolysaccharides, known as T-cell-independent antigens, and the cell switches class production to either IgG, IgA or IgE class antibodies. IgG is the major antibody component and can neutralize toxins, activate the complement system (Fig. 3), and react with pathogens. IgA is the principal antibody of mucosal immunity and is found in external secretions such as tears, milk, nasal and intestinal secretions. IgE is the principal antibody of allergic responses and immune reactions to parasites. IgD is found on the surface of immature B cells but its function remains obscure.

Any particular B cell produces one specific antibody, whose variable region recognizes one specific antigen (Fig. 4). The fact that an almost infinite range of specificities can be encoded by a finite number of genes is explained by the fact that during B-cell development in the bone marrow, gene segments are randomly irreversibly joined so as to encode the variable region of the antibody for that particular B cell. Through the recombination of various germline gene segments—grouped into V, D and J segments—one of over 10^{14} potential different genetic combinations is produced in each individual B lymphocyte. When a particular B-cell antibody molecule binds antigen, it is engulfed and presented to other lymphocytes by means of the MHC class II molecule of the B lymphocyte. Since B cells can present antigen to T lymphocytes in their MHC class II molecule peptide groove, B cells are considered to be a type of APC. With the right response from the interacting “helper” T cell, the B cell undergoes clonal expansion and begins to secrete antibody with the same specificity.

Another subset of lymphocytes matures in the thymus and carries the T-cell receptor (CD3), which allows these cells to recognize specific peptides presented by APCs within the peptide groove of either MHC class I or II molecules. T cells are responsible for adaptive cellular immunity. Like antibodies, T-cell receptors that recognize over 10^{18} specific peptides in specific MHC molecules are produced by random combinations of the germline V, D, and J segment genes that make up the variable regions of the T-cell receptor. These cells undergo

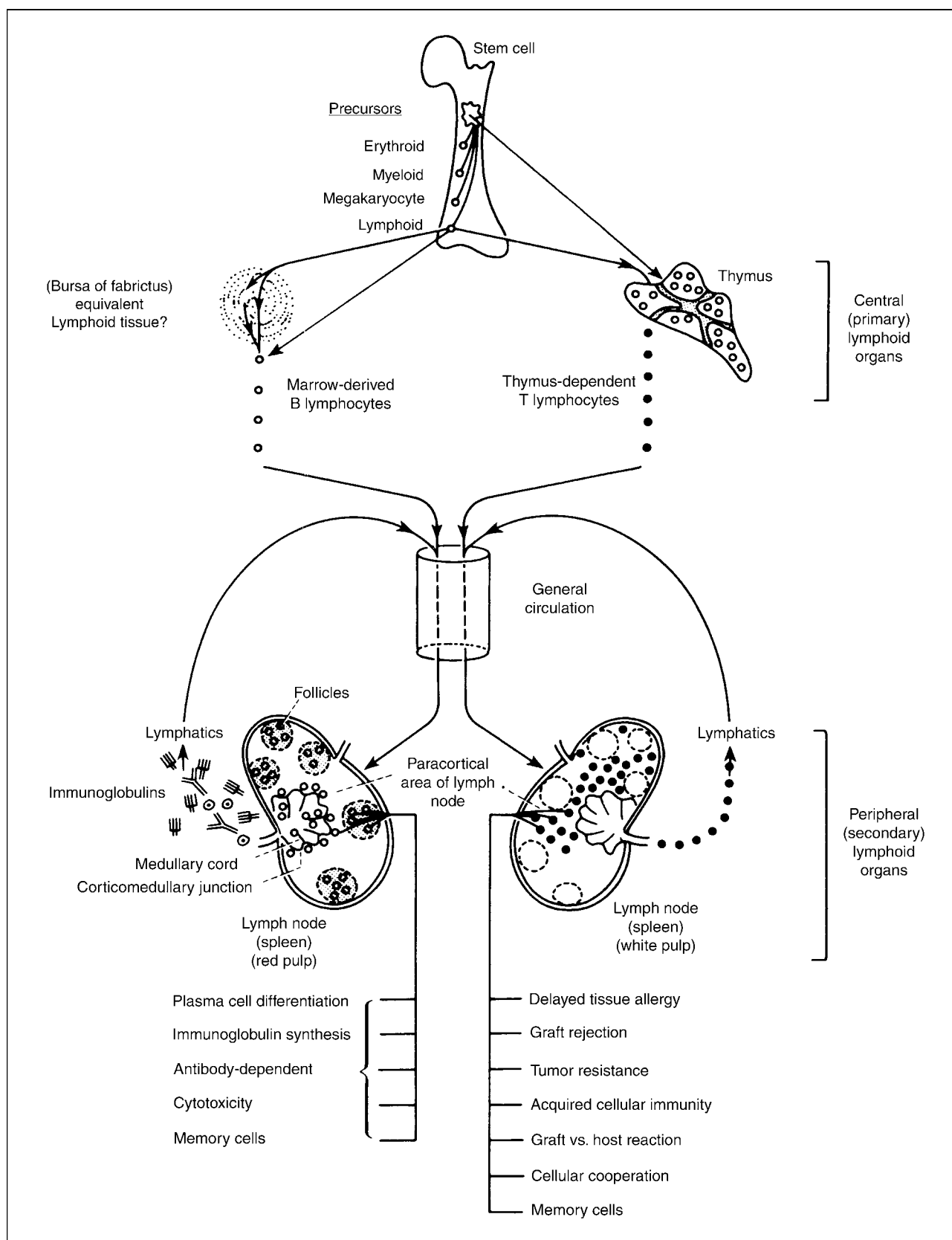


Figure 2 Origin and development of the lymphoid system. The peripheral lymphoid organs include, in addition to lymph nodes, the spleen and lymphocyte aggregates in the submucosal tissue of the gut and elsewhere in the body. *Source:* Courtesy of Dr. Amjad H.S. Rahi.

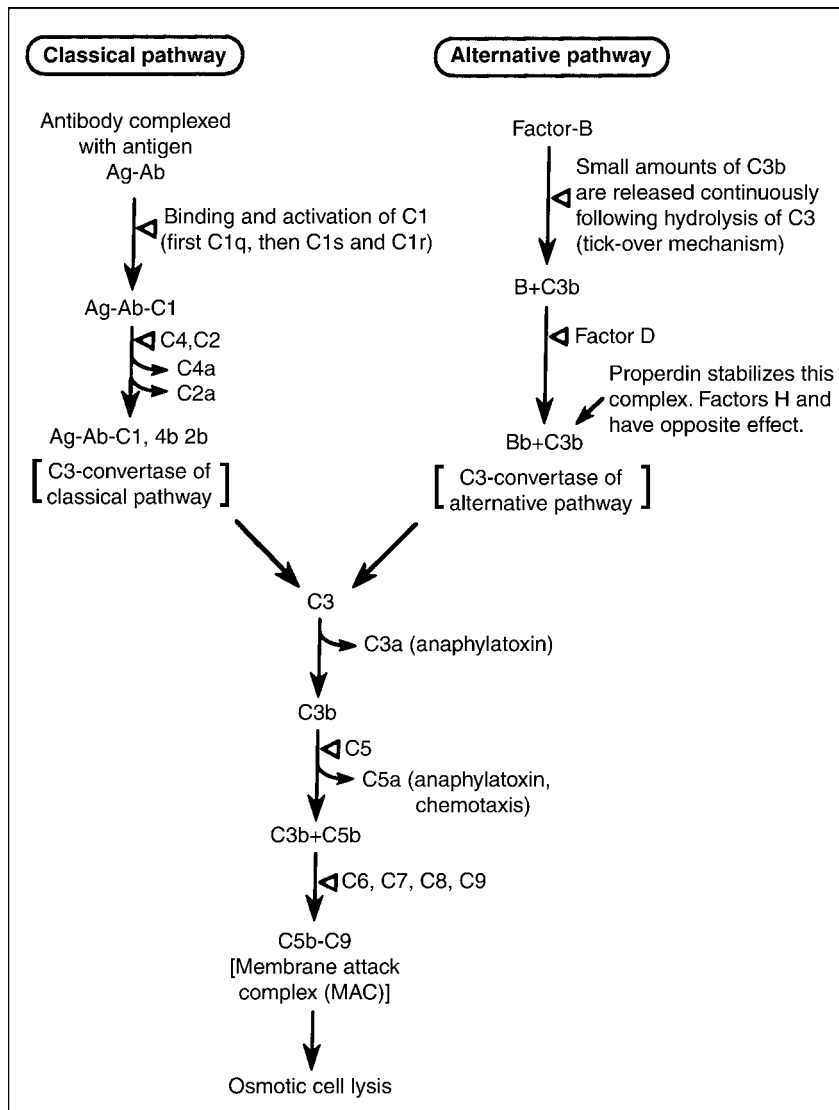


Figure 3 Complement activation. The classic pathway generally requires an antigen–antibody reaction on the surface of the target cell (although C-reactive protein can also be effective), whereas the alternative pathway can be activated by aggregated immunoglobulins (such as IgG) and proteolytic enzymes. C3-convertase generated by either of the pathways leads to the sequential activation of C3 through C9. The production of a membrane attack complex results in permeability damage to the phospholipid layer of the cell membrane by inserting molecules of C9 in a concentric fashion to create a tunnel. This predisposes to osmotic lysis of the target cell by permitting the entry of sodium ions. *Source:* Courtesy of Dr. Amjad H. S. Rahi.

clonal deletion during maturation in the thymus which is thought to remove all T cells that react with the host's own antigens, so as to preclude potentially self-destructive T-cell activation (i.e., autoimmunity). When mature T cells are presented with an antigen for which they have a specific T-cell receptor (CD3) by APCs, the T cells undergo clonal expansion, initiating an immune response targeted at the specific antigen. CD3 was first identified using monoclonal antibodies that bound only one cloned T-cell line but not others thereby inhibiting antigen recognition by that clone of T cells, or specifically activating them. Each receptor consists of two different polypeptide chains, termed the T-cell receptor α and β chains, linked by a disulfide bond. These heterodimers are analogous in structure to the Fab fragment of an Ig molecule, and they account for antigen recognition by most T cells. A minority of T cells bear an alternative, but structurally similar, receptor made up of a different pair of

polypeptide chains designated γ and δ . These T-cell receptors appear to have different antigen-recognition properties from the α and β T-cell receptors, although the function of γ and δ T cells in immune responses is not yet well understood. Each T cell carries approximately 30,000 CD3 molecules, but all CD3 molecules on any given cell express only one of over 10^{18} V, D and J segment combinations, rendering each T cell specific for a single antigen presented in the context of an MHC molecule. The CD3 molecule is the only antigen-specific molecule on the T cell. Subsets of T cells react to antigen in two different ways depending on the co-receptor molecule (either CD4 or CD8) associated with their T-cell receptor (CD3). Cells bearing the CD4 co-receptor promote the immune response through secreted cytokines that activate surrounding lymphocytes and/or macrophages. These cells recognize extracellular antigens that have been processed in intracellular vesicles following

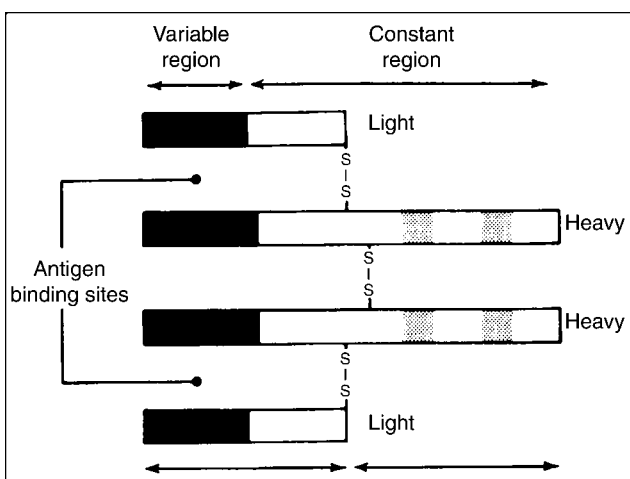


Figure 4 Immunoglobulin G (IgG) molecule. The antigen-binding site is located at the amino termini of the light and heavy chains and has a variable composition. The chains are linked together by disulfide bonds; similar internal bonds form loops that in the Fc region of the heavy chain provide sites for the several biological activities of the molecule. In the IgG molecule there are two such sites on the constant region of the heavy chain: C_H2 is concerned with complement fixation and regulation of catabolism and C_H3 with macrophage binding. C_H1 is located on the constant region of the heavy chain within the Fab part of the molecule. *Source:* Adapted from Garner A. The immune response. In: Scadding JG, Cumming G, eds. Scientific Foundations of Respiratory Medicine. Heinemann: London, 1981.

ingestion by an APC and presented as peptide in the groove of MHC class II molecules. T cells bearing the CD8 co-receptor are cytotoxic, destroying the cells harboring the antigen. These cells recognize cytotoxic antigens, such as viral antigens, presented in the groove of MHC class I molecules and are called cytotoxic T cells. In addition to antigen-specific signals mediated through the T-cell receptor and co-receptor molecules, T cells also require antigen-nonspecific costimulation for activation. The B7 family of molecules on APCs, which include B7-1 (CD80) and B7-2 (CD86), play important roles in providing costimulatory signals required for development of antigen-specific immune responses.

A third type of lymphocyte is the natural killer (NK) cell which plays an important role in innate or antigen-nonspecific cytotoxic responses to tumor cells or virus-infected cells. The cells are large granular lymphocytes and are characterized by the lack of CD3 and Ig on their surface. NK cells can kill a variety of targets, including tumor cells, without the need of specific antigen interaction. Recent reports suggest that the NK-cell population is heterogeneous, for although most are CD3⁻, CD16⁺, and CD56⁺, a small proportion may also express CD3 antigen (28). They do not require IgG to kill the target. It has been demonstrated that the granules of NK cells contain a

protein called perforin, which resembles the ninth component of complement (C9) and produces anatomical holes (i.e., permeability defects) in the target cells (70). NK cells are CD56⁺ and are activated in the presence of interferon gamma (IFN γ) (28,30).

ANTIGEN

An antigen is classically defined as any substance that can bind to a specific antibody; this includes proteins, carbohydrates and nucleic acids. All antigens by definition have the potential to react with specific antibodies, but some need to be attached to a protein to successfully elicit an immune response. Any substance that elicits an immune response is termed immunogenic and is called an immunogen. Many non-protein antigens are not immunogenic unless they are presented in conjunction with a protein immunogen carrier and it is the immunogenicity of the protein antigens that determines the immune response in most cases. Furthermore, although almost any molecule can be recognized by antibody as an antigen, usually only proteins elicit fully developed adaptive immune responses, because proteins have the unique ability to be recognized by CD3 of T cells, which contribute to most antibody responses and are required for immunologic memory. An adaptive immune response that includes immunologic memory can be induced by non-protein antigens, only if these antigens are attached to a protein carrier which can engage the T cell.

Most proteins are poorly immunogenic or non-immunogenic when administered by themselves in experimental models. Significant adaptive immune responses to protein antigens usually require that the antigen be injected in a mixture known as an adjuvant, defined simply as any substance that enhances the immunogenicity of substances mixed with it. Unlike protein carriers, adjuvants do not form stable linkages with the antigen. Many adjuvants increase macrophage phagocytosis of antigens and delay their release so as to increase the duration of immune exposure. These are thought to be the main functions of the oil-in-water emulsion produced by the use of incomplete Freund adjuvant. Complete Freund adjuvant additionally contains dead mycobacteria which induce IL12 and costimulatory molecules and thereby render macrophages more effective APCs (24). The magnitude of the immune response to an immunogen also depends on the dose of immunogen administered. At very low doses, too few immune competent cells are engaged to produce a significant response. As the dose of immunogen is increased, the immune response increases in severity until a plateau level is reached. At very high doses of immunogen the immune response is often inhibited by a process

termed tolerance. Although many autologous proteins of the host can be antigenic, they are seldom immunogenic because of multiple mechanisms by which the immune system becomes tolerant to self-antigens (these mechanisms are discussed in greater detail in the section on Immunologic Tolerance). A breakdown in the mechanisms of tolerance to self-antigens causes autoimmune disease. Many autologous ocular antigens have been described, the best characterized of which is S-antigen, isolated from the retina, and now known to be a protein called arrestin found in rod photoreceptors and involved in the phototransduction cascade. In numerous species, if S-antigen is injected subcutaneously with complete Freund adjuvant, a breakdown in the physiologic tolerance to S-antigen occurs, and the animal develops experimental autoimmune uveitis (EAU) (see Chapter 4).

IMMUNE RESPONSE

The recognition of antigen by T cells and the resulting response of these and other cells of the immune system can be divided into the afferent and effector immune responses. The afferent immune response includes antigen-nonspecific activation of the immune response via complement components, NK cells, and PMNs and antigen-specific activation via antibody and antigen presentation to T lymphocytes. The antigen nonspecific process, or innate immunity, occurs primarily in the involved tissue itself. This process is immediate and responds to a relatively small number of evolutionarily conserved antigens such as bacterial lipopolysaccharide, other bacterial cell wall components, and exotoxins (29). By contrast, adaptive immunity involves antigen recognition by lymphocytes, typically requires days to establish an effective clonal expansion, and is localized primarily in lymphoid tissues, such as are present in the conjunctiva, in draining lymph nodes or the spleen. In general, presentation of antigen in non-splenic lymphoid tissue results in the development of a delayed type of immune response with immunologic memory. The primary response results from the initial contact with the antigen. Secondary, tertiary and subsequent responses are characterized by increasing magnitude. Repetitive challenge with antigen to achieve a heightened state of immunity is called hyperimmunization, and is the basic strategy of clinical immunization.

The spleen is a special lymphoid organ, in so far as it serves as the central lymph node of the circulatory system. The direct exposure to circulating antigen may predispose the spleen to suppress immune responses and may account for some of the spleen-dependent immune deviation seen in response to antigens injected in the anterior chamber which gain direct exposure to the venous circulation via aqueous humor outflow (7).

THE AFFERENT IMMUNE RESPONSE

The ocular surface is perhaps the most important site for innate immunity in protecting the eye from exogenous insults. Intact surface epithelium, low surface temperature, neutral pH of the normal tear film, tear-borne macrophages, and an intact blink response are important first-line components of ocular immunity. Tears wash away antigens and contain lactoferrin and lysozyme (both bacteriostatic), secretory IgA, IgG and complement, all of which are derived from lymphocytes lying beneath the conjunctival epithelium and in the stroma of the lacrimal gland. Lactoferrin upregulates the production of granulocyte and macrophage-derived colony-stimulating factor (51). Lysozyme can lyse the cell walls of some Gram-positive bacteria and can enhance bacteriolysis by secretory IgA (55). The submucosal cells of the conjunctiva contain mast cells which can release histamine, heparin, platelet activating factor, and leukotrienes, that cause vasodilation and increased vascular permeability, promoting further local inflammation. The resulting transudate may contain bacteriocidal substances such as specific antibodies and complement.

In the conjunctiva, localized lymphoid aggregates, called conjunctival-associated lymphoid tissue (CALT) function to mount adaptive immune responses to antigens collected in the ocular surface. Similar large aggregates of mucosal lymphoid tissue are found along the gastrointestinal and bronchial tracks from the tonsils and adenoids to the appendix. Peyer patches of the small intestine are important as sites of induction of mucosal immunity. The surface epithelium covering Peyer patches lacks goblet cells and allows for maximal exposure to epithelial cells to the intestinal contents. Specialized epithelial cells called M cells serve to take up molecules and particles from the gut lumen and transport them across an epithelium within intracellular vesicles and release them into the extracellular space on the basal side of the cell (transcytosis). Nearby APCs then process these molecules and display the antigen to surrounding lymphocytes. The epithelium of the ocular surface overlying CALT also lacks goblet cells, and the lymphoid aggregate contains T cells and B cells and stain positive for IgA (15). Recent evidence suggests that M cells are also present in CALT and function to transcytose antigen from the ocular surface to underlying APCs (26).

In the eye and its adnexal structures much of the afferent arm of the immune response occurs in specialized immune organs. Lymphatic vessels are found predominantly in the eyelids. They are arranged in pre-tarsal and post-tarsal plexuses and are connected by cross-channels. The post-tarsal plexus drains the

conjunctiva and meibomian glands, while the pre-tarsal plexus drains the eyelid skin. The lateral aspects of both plexuses drain into the pre-auricular and parotid lymph nodes, while the medial aspects drain into the sub-mandibular lymph nodes (55). Although, when vascularized the cornea may form some lymphatic channels (54), the remainder of the ocular anatomical structures, with the exception of the conjunctiva and eyelid, do not contain lymphatic channels. Nevertheless, extracellular fluid may drain through perivenous spaces of the posterior segment, through the lamina cribosa, and into the spaces of the optic nerve (56). There is evidence that antigen injected intravitreally reaches the pre-auricular lymph node (18).

Antigen and lymphocytes classically encounter each other in peripheral lymphoid organs (Fig. 1). Naïve lymphocytes circulate through the circulatory system and lymphoid tissues. Specialized APCs such as dendritic cells, macrophages and B cells that have engulfed antigen by means of surface antibodies, display antigen to these circulating lymphocytes in the peptide groove of their MHC class II molecules. Lymph nodes are highly organized structures located at the points of convergence of lymphatic channels of the lymphatic system, which collect extracellular fluid and return it back to the venous circulation. Antigen and APCs collect in the lymph nodes. Collections of B lymphocytes form follicles nearest to the afferent lymphatic vessels (Fig. 5). A neighboring paracortical area is predominantly composed of T lymphocytes.

Finally, closest to the efferent end of the lymph node are structures termed medullary cords which are rich in macrophages and antibody producing B lymphocytes. When naïve lymphocytes encounter antigen specific for their surface antibody or T-cell receptor they stop migrating and transform into lymphoblasts, with enlarged nuclei and cytoplasm, synthesize new RNAs and proteins, and begin to divide over 4 to 5 days. It is through this process that one naïve lymphocyte can give rise to numerous daughter cells, each with specificity to the same antigen. Naïve B cells give rise to plasma cells, and naïve T cells become activated helper or cytotoxic/killer T cells. Although lymphocytes may become activated locally in the choroid, there is evidence that at least in the case of sympathetic ophthalmia (SO) drainage of ocular antigens to the local conjunctival lymphoid tissue is necessary for the development of the immune response (45).

A large proportion of cardiac output continuously filters through the spleen, where it passes by lymphocytes, macrophages and dendritic cells that respond to infectious agents, abnormal erythrocytes or leukocytes, and blood-borne pathogens. Splenectomy in adults is well tolerated but results in increased circulating malformed erythrocytes and an increased risk of sepsis due to pyogenic bacteria. Some mechanisms of ocular immune response, such as the deviated response to anterior chamber or vitreous antigens, appear to be dependent on the spleen. It has been postulated that in the mouse model of

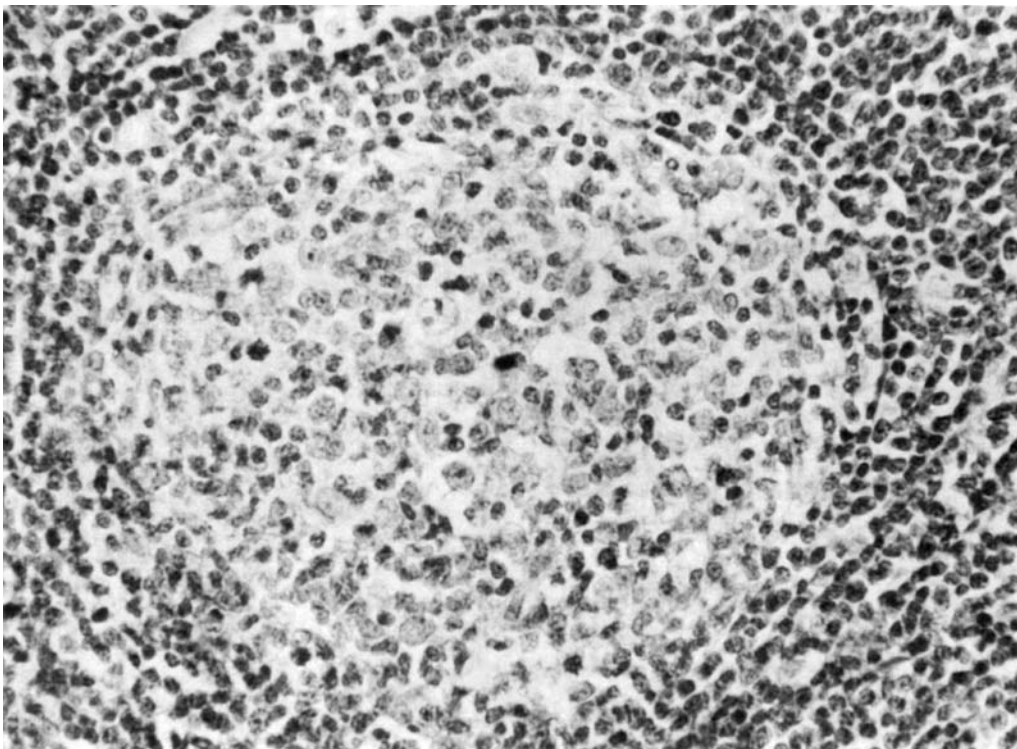


Figure 5 Lymphoid follicle with a germinal center occupied by pale-staining B lymphoblasts, some dendritic reticulum cells and macrophages (hematoxylin and eosin, $\times 195$). *Source:* Courtesy of Dr. Amjad H.S. Rahi.

intraocular immune response, known as anterior chamber-associated immune deviation (ACAID), local APCs in ocular tissue internalize antigen introduced experimentally in the anterior chamber. These APCs are then thought to migrate from the eye via aqueous humor outflow to the systemic circulation and ultimately to the spleen. Through their interaction with splenic T cells, B cells and NK cells, they induce differentiation of CD8+ and CD4+ regulatory T cells resulting in a cytotoxic immune response and a delayed type of T-cell response. This pathway has been proposed by Streilein (59) although transportation of antigen by APCs from the eye to the spleen has never been directly demonstrated.

EFFECTOR IMMUNE MECHANISMS

Antibodies are Y-shaped molecules, the arms of which form two identical antigen-binding sites (Fig. 4). They are highly variable from one molecule to another, providing the diversity needed to encounter 10^{14} different potential antigens through random rearrangement of germline genes as discussed above. The stem of the Y defines the class or isotype of antibody, of which there are five: IgM, IgG, IgA, IgE, and IgD. Each of the five classes engages a distinct set of effector mechanisms once antigen is recognized. Antibodies can neutralize pathogens by binding to them and thereby inhibiting their pathologic function. Antibodies also can coat pathogens such as bacteria and trigger phagocytes to engulf these antibody-coated bacteria; this process is known as opsonization. Some subtypes of antibodies also activate complement which further enhances opsonization and leads to destruction of pathogens by the formation of the membrane-attached complex of complement. IgE antibodies in contact with antigen can crosslink membrane-bound IgE receptors on mast cells and lead to cell activation and release of preformed mediators, such as histamine, proteoglycans, neutral serine proteases and carboxypeptidase, from intracytoplasmic granules, initiating a response, recognized as an allergic response.

Pathogens are only accessible to antibodies in the blood and extracellular spaces. However, some pathogenic bacteria and parasites, and all viruses live intracellularly and cannot be detected by antibodies. Cytotoxic CD8+ T cells can react to specific peptide

sequences derived from intracellular pathogens, particularly viral peptides, which are expressed on the host cell in the groove of MHC class I molecules. These cytotoxic T cells then destroy the infected cell, and thereby help clear the intracellular pathogen. CD4+ T cells can be divided into two groups: Th1 and Th2 helper T cells (Table 2 and Fig. 6). Th1 cells are potent clearers of intracellular bacteria such as *Mycobacterium tuberculosis* and *M. leprae* by activating macrophages to fuse bacteria containing vesicles with lysozymes. They also secrete cytokines that stimulate macrophage migration to the site of infection. Th2 helper T cells activate B cells to secrete antibodies, and drive humoral immunity. The term "helper" T cell is often used to describe both Th1 cells and Th2 cells since Th1 cells "help" activate macrophages and Th2 cells "help" activate B cells. The term "helper" T cell was used traditionally for T cells that activate B cells, and according to some authorities should thus be reserved for Th2 cells. Th2-cell differentiation is driven by IL4, IL5, IL6, IL10, and IL13. IL4 and IL13 in particular can stimulate Ig class switching, leading to the production of IgA, IgE or IgG.

The differentiation of naïve CD4+ T cells into Th1 cells or Th2 cells occurs at the draining lymph node, and determines whether the immune response will be characterized by macrophage activation (Th1) or antibody production (Th2). Surrounding cytokines have a profound effect guiding this differentiation. IL12 and IFN γ tend to promote Th1 response, while IL4 and IL6 promote Th2 differentiation. Additionally, cytokines released by either Th1 cells or Th2 cells inhibit the differentiation of the other subtype, i.e., both subtypes promote their own differentiation and inhibit the differentiation of the other subtype. The differential capacity of pathogens to interact with dendritic cells, macrophages, and NK cells influences the overall balance of Th1 versus Th2 cytokines.

IL12, produced by dendritic cells and macrophages, and IFN γ , produced by NK cells and CD8+ T cells, are seen in the early phase of the response to viruses and some intracellular bacteria, such as *Listeria* sp. This cytokine milieu leads to Th1 differentiation. In contrast, IL4 is produced by a special set of NK cells called NK1.1+ T cells which thereby select the Th2 response. The path of Th differentiation greatly effects the host response to pathogen.

Table 2 Characteristics of Th1 and Th2 Cells

CD4 T-cell subsets	
Helper Th1 cells	Helper Th2 cells
Synthesize cytokines: IL2, IFN γ , TNF β , IL12	Synthesize cytokines: IL4, IL5, IL10
Inhibit Th2-cell differentiation	Inhibit Th1-cell differentiation
Promote B-cell IgG1 and IgG3 production	Promote B-cell IgE and IgA production
Promote CD8+ T-cell response	Promote antibody mediated response

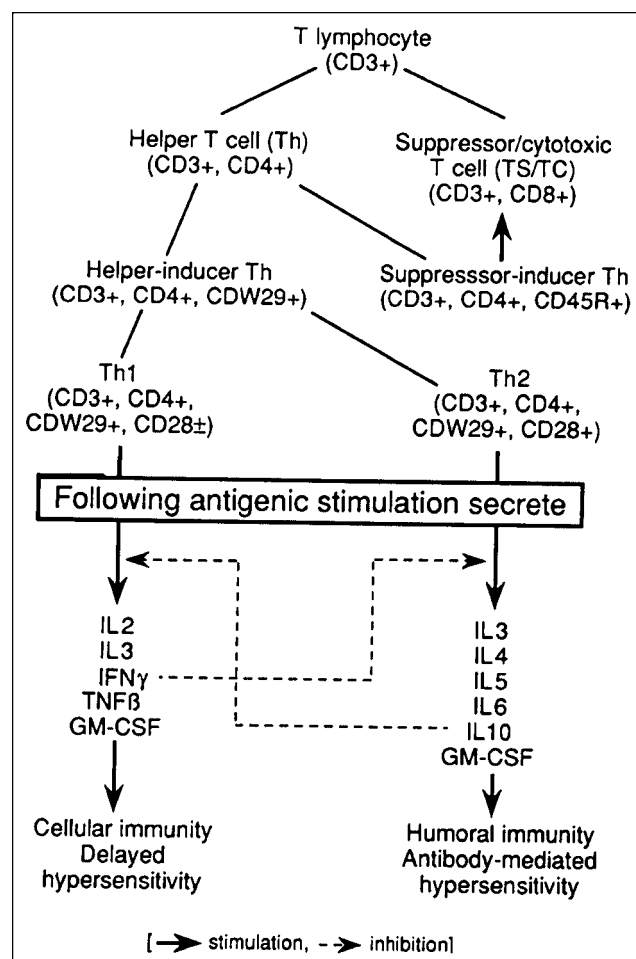


Figure 6 Phenotypic and functional heterogeneity of T lymphocytes: roles in humoral and cellular immune responses. Th1 mediate cellular immunity by activating cytotoxic T cells, K cells, and macrophages while suppressing humoral immunity through interferon γ ($\text{IFN}\gamma$). Th2 secretes a variety of interleukins that stimulate B cells and promotes humoral immunity. Th1, through the mediation of $\text{IFN}\gamma$, can also suppress Th2 activity, whereas IL10 derived from Th2 activity is inhibitory to Th1 function. Source: Courtesy of Dr. Amjad H.S. Rahi.

For example, Th1 cytokines are associated with tuberculoid leprosy, while Th2 cytokines are associated with the more severe lepromatous leprosy resulting from Th1 suppression and inability of the host to activate macrophages (32). Similarly, a mutation in the *IFNG* gene on chromosome 12 (12q14) that encodes for $\text{IFN}\gamma$ causes increased susceptibility to mycobacterial infection (37). Experimental models such as EAU indicate that most posterior uveitis is predominantly driven by Th1 response (8).

IMMUNOLOGIC TOLERANCE

There are several inbuilt mechanisms of the host which eliminate T cells and B cells reactive to self-antigens so that the host tissue is not destroyed by the immune

process. This self-tolerance depends on central and peripheral tolerance mechanisms. Central tolerance to self-antigens is primarily acquired by clonal deletion of developing T lymphocytes in the thymus. T cells that recognize ubiquitous intracellular proteins are clonally deleted so that they may not direct immune responses toward self-antigens. This process occurs in two steps. First, T cells that do not recognize the host's MHC molecules are eliminated, in a process termed positive selection, because cells that recognize the MHC molecules are selected. Second, cells that encounter antigen during this developmental stage undergo cell death. A majority of T cells consequently undergo apoptosis (see Chapter 2). This second process is termed negative selection or clonal deletion. The combination of these two processes yields mature T cells that are MHC restricted, but not highly reactive with autologous antigen. B cells undergo similar negative selection in the bone marrow as well.

A number of mechanisms exist to induce tolerance in peripherally circulating lymphocytes. These include immunologic ignorance, anergy and T-cell mediated active suppression. Immunologic ignorance refers to the inability of low concentrations of low avidity self-antigens to illicit an immune response, even if peripheral T cells with the appropriate specificity are present. For example, under normal circumstances circulating T cells do not encounter myelin basic protein (MBP) in high enough concentrations to illicit an immune response. When these animals are immunized with a high concentration of MBP peripheral T cells become activated, migrate into the peripheral nervous system and CNS, and cause experimental encephalomyelitis. Anergy is tolerance to self-antigens found in higher concentrations but presented to T cells in the absence of costimulatory signals. The T cell is rendered inactive but not eliminated. The expression of appropriate costimulatory signals is often dependent on the context of antigen presentation. For example, cytokines from nearby PMNs or NK cells that have been activated in an antigen-independent manner, can cause certain costimulatory molecules to be upregulated on local APCs. Antigen presented in the absence of this environment may produce anergy.

Some T cells, first termed suppressor T cells and more recently termed regulatory T cells, can suppress other lymphocytes via cytokines such as transforming growth factor beta ($\text{TGF}\beta$) or IL10 (39). A subset of IL10 producing T cells can be induced by repeated intranasal administration of an antigenic peptide of MBP. These regulatory cells inhibit the proliferation of naïve MBP-specific T cells *in vivo* and *in vitro*. Also a subset of activated T cells expressing the alpha chain of the IL2 receptor (CD25) has been shown to actively suppress the immune response. These $\text{CD4}^+ \text{CD25}^+$

T cells make up 5% to 10% of the CD4⁺ T cells in healthy adult mice and humans and have been shown to control various aspects of autoimmunity following neonatal thymectomy.

AUTOIMMUNITY AND INDUCTION OF TOLERANCE

The breakdown of tolerance leads to the development of autoimmune disease (48). It remains unclear why the breakdown occurs. Genetic predisposition seems to play an important role in the development of autoimmune disease. Patients often describe a family history of autoimmune disease. In most cases the likelihood of developing disease correlates with the degree of shared HLA haplotypes. It has become clear that in many autoimmune diseases, HLA haplotype is the best predictor of disease. Other genes, for example, encoding proteins involved in the immune response may predispose to autoimmunity. For example, mutations in the *CTLA4* gene on chromosome 2 (2q33) that encodes for a costimulatory molecule expressed by activated T cells, have been found to influence susceptibility to diabetes mellitus and thyroiditis (60). However, studies of monozygotic twins suggest that heredity accounts for only about one-third of the risk of developing autoimmune disease (6,11). Some of the lack of concordance in monozygotic twins may be a consequence of post-germline recombination of genetic elements seen in the production of immune competent proteins such as the variable regions of antibodies or CD3. The apparently random processes produce differing immune systems in genetically identical individuals. Environmental agents are also known to play a role in the development of disease. Best characterized is the ability of certain drugs to induce autoimmune disease, such as lupus erythematosus-like disorders caused by procainamide or hydralazine, or hemolytic anemia associated with α -methyl dopa. Apoptosis plays an important role in autoimmune disease (see Chapter 2).

Infections are widely thought to play a prominent role in eliciting autoimmune disease, perhaps best characterized in the development of rheumatic fever and rheumatic heart disease following infection with beta hemolytic streptococci. Cross-reaction of immune responses directed at the infectious organism with autologous proteins has been shown to cause autoimmune disease in experimental models, this molecular mimicry has been shown in only a few human diseases. Alternatively, infectious organisms may promote autoimmunity by increasing immunologic exposure to autologous proteins, a process termed the bystander effect. Another aspect of autoimmune disease that is not well understood is the common predilection of women to develop many autoimmune diseases. It has been postulated that sex differences in

the hypothalamic–pituitary–adrenal axis and steroid hormones, including estrogens, play important roles in regulating antibody production and T-cell regulation. These interactions remain unclear.

Manipulating the immune response to induce tolerance in experimental models of autoimmune disease is a hopeful strategy for treating autoimmune disease. For example, experimental autoimmune encephalitis is caused by a Th1 response to MBP. Oral administration of the protein produces a Th2, IgA response in a mouse model and inhibits Th1 disease. This mechanism of tolerance induction has been exploited in EAU as well. Animal studies have shown that the oral administration of either whole arrestin (S-antigen) or its peptide derivatives can suppress EAU. Human studies have shown that in a select group of uveitis patients that demonstrate robust T-cell proliferative responses to arrestin, administration of oral arrestin results in a reduced necessity for systemic immunosuppression as compared to controls (38). Nasal administration of retinal extract, including arrestin, has also proven effective in inducing tolerance in animal models (12).

IMMUNOPATHOLOGIC RESPONSES

In 1963, Gell and Coombs (16) proposed a scheme of classifying unrestrained beneficial immune responses into four types of hypersensitivity reactions. Although much has been learned about the molecular characteristics of the immune response since 1963, these four types of hypersensitivities as described in 1963 still provide a useful framework to characterize immune pathways.

Hypersensitivity type I is characterized by antigen-specific IgE binding to mast cells, which initiates an allergic response. IgE is produced locally or by plasma cells in draining lymph nodes (Fig. 7). Class switching from IgM to IgE is driven by CD4⁺ Th2 cells. Immune cells at mucosal sites are thought to be predisposed to secreting IL4 and other Th2 cytokines. Specialized CD4⁺ T cells, called NK1.1⁺ cells, are also known to participate in the generation of hypersensitivity type I and are known to produce abundant IL4. Very low doses of antigen presented at the mucosal surface favors the activation of a Th2 response.

The immediate allergic reaction is caused by mast cell and basophil degranulation, particularly by release of intracellularly stored mediators, such as histamine, tryptase, and cytokines. Conjunctival mast cells have been shown to produce IL4, IL5, IL6, and TNF α , thereby further driving Th2 differentiation, and consequently IgE production. Late phase reactions in the conjunctiva are rare and include increased Th2-cell involvement, and eosinophil recruitment and activation. Activated eosinophils release basic, highly

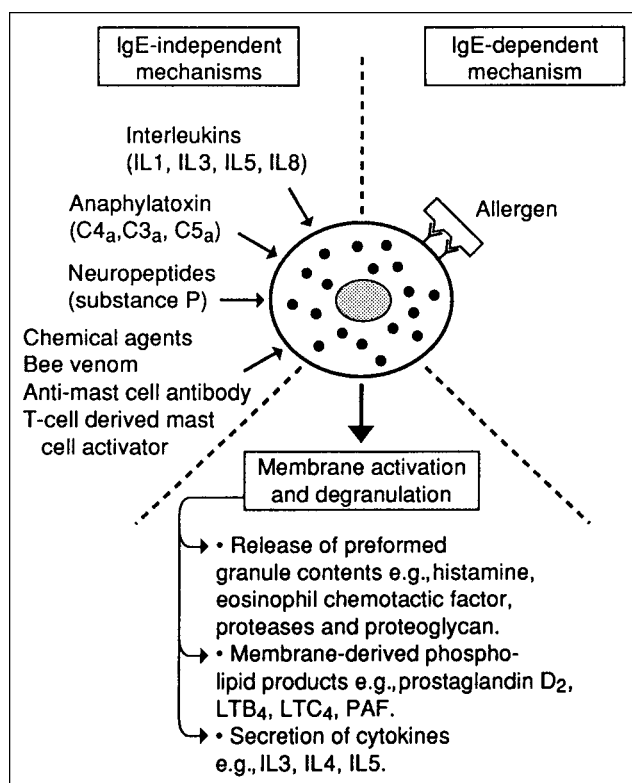


Figure 7 Hypersensitivity type I reaction. Mast cells (and basophils) are activated when an allergen binds to at least two IgE molecules on the cell surface. Consequent degranulation incurs the release of preformed histamine, enzymes, and chemotactic and possibly other factors. Plasma membrane activation promotes the synthesis and release of prostaglandins, particularly PGD₂ in the case of mast cells, and leukotrienes C₄, D₄, and E₄. Mast cells can also be degranulated in the absence of an allergen by certain interleukins, by anaphylatoxin fragments (C4a, C3a, and C5a) of activated complement, by neuropeptides, such as substance P, and by a number of other disparate mechanisms. *Source:* Courtesy of Dr. Amjad H.S. Rahi.

charged polypeptides, including major basic protein, eosinophilic-derived neurotoxin and eosinophilic peroxidase. While these late phase reactions are not thought to play a prominent role in the more common forms of allergic conjunctivitis, such as seasonal or perennial conjunctivitis, they may play a significant role in vernal keratoconjunctivitis and atopic keratoconjunctivitis (see Chapter 5).

Hypersensitivity type II is characterized by antibody-mediated complement, NK-cell and/or macrophage cytotoxicity (Fig. 8). The classic example of this type of hypersensitivity is the clearance of erythrocytes from the circulation after antibodies react to a drug that is bound to the erythrocyte surface. The antibodies then react with the antibody receptor on macrophages, thereby activating them to phagocytose the erythrocytes. Both ocular cicatricial pemphigoid (see Chapter 5) and lens-induced uveitis (see Chapter 4) may involve hypersensitivity type II

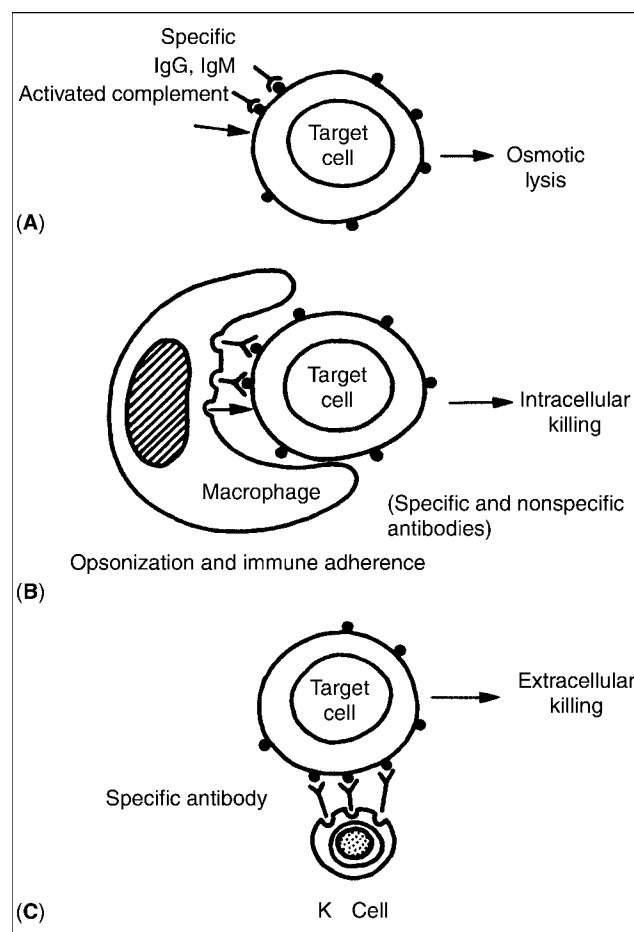


Figure 8 Antibody- and complement-mediated hypersensitivity type II. (A) Binding of IgG and IgM to antigenic determinants on the surface of the target cell activates the cytolytic components of the complement system. (B) Facilitation of phagocytosis is provided by antibody bound to the target cell (opsonization); phagocytosis is further enhanced by the presence of C3b (immune adherence) since macrophages have receptors for both IgG and C3b. (C) The target cell can be attacked by killer lymphocytes provided it is coated with specific antibody. Macrophages may also contribute to K-cell type of activity. *Source:* Courtesy of Dr. Amjad H.S. Rahi.

through immune responses mediated by anti-conjunctival basement membrane antibodies and anti-lens protein antibodies, respectively. Other examples include transfusion reactions and myasthenia gravis.

Hypersensitivity type III reactions are directed against soluble antigens (Fig. 9). The pathologic state is caused by inefficient removal of immune complexes (i.e. antibody-antigen complexes) and their subsequent deposition within blood vessel walls and other tissues. The pathogenicity of immune complexes is determined by their size and the amount, affinity and isotype of the antibody. Larger complexes fix complement and are readily cleared by phagocytes. Smaller complexes formed in antigen excess are less readily cleared and remain deposited in blood vessel walls, where they can

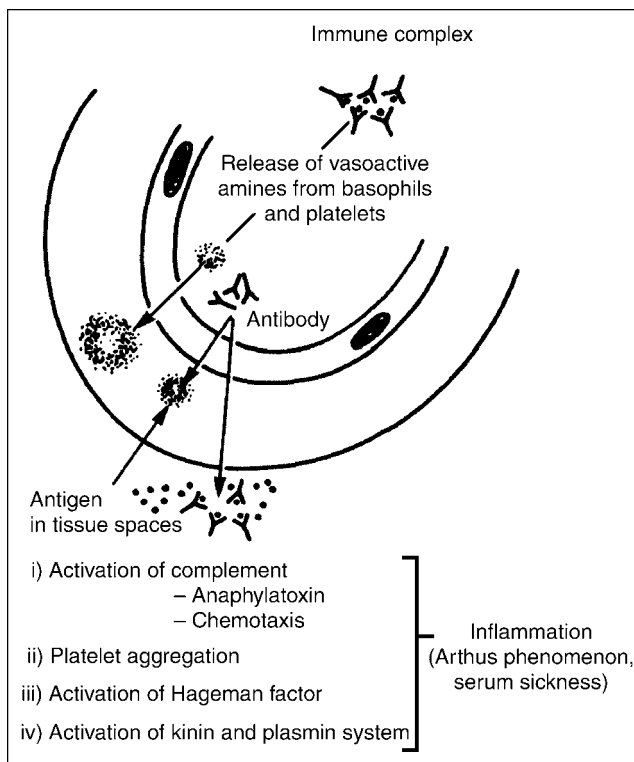


Figure 9 Immune complex-mediated hypersensitivity type III. Circulating immune complexes enter the vessel wall, aided in doing so by an increase in vascular permeability by vasoactive amines released from basophils and platelets. Deposition of immune complex (or its in situ formation in an Arthus-type reaction) is followed by activation of complement, leukocytic infiltration, and necrotizing inflammation in the vessel wall. *Source:* Courtesy of Dr. Amjad H.S. Rahi.

activate various leukocytes via the antibody receptor. The Arthus reaction is a localized cutaneous reaction to subcutaneous antigen to which the host has IgG antibodies. Local deposition of immune complexes causes activation of mast cells and other leukocytes via the antibody receptor. The local inflammatory response increases vascular permeability and calls in PMNs and other immune cells, leading to local clinically observed inflammation. These mechanisms are involved in phacoanaphylaxis, systemic lupus erythematosus (SLE), Behçet disease, Wegener granulomatosis and other causes of scleritis and retinal vasculitis (Fig. 10) (see Chapters 4–6). Immune complexes are detected in conjunctival specimens from selected cases of anterior segment manifestations of SLE (19). At least one animal model of necrotizing scleritis demonstrates a deposition of immune complexes (20). The role of immune complexes in retinal vasculitis is not clear. While circulating immune complex titers correlate with the severity of idiopathic retinal vasculitis in patients with Behçet disease, absence of circulating immune complexes was associated with more severe retinal vasculitis (23). It may

be then that some immune complexes are protective, potentially by absorbing pathogenic antibodies.

Hypersensitivity type IV reactions are delayed-type hypersensitivity reactions mediated by effector T cells (Fig. 11). Because the T lymphocyte is the driving force behind these reactions, this type of hypersensitivity can be transferred from one animal to another by transferring a reactive population of T cells, a process termed adoptive transfer. The prototypic type IV response is the tuberculin skin test. Intradermal injections of *M. tuberculosis* derivatives causes a Th1 response which results in localized inflammation 24 to 72 hours after inoculation, if the individual has *M. tuberculosis*-specific T cells. This mechanism is thought to be central in Th1-mediated diseases such as EAU, SO, Vogt-Koyanagi-Harada disease (VKH), Behçet disease, and sarcoid related uveitis (35) (see Chapters 4 and 6). Additionally, delayed type hypersensitivity can be seen with Th2-related responses. Hypersensitivity type I requires T cell help for class switching to IgE. Thus all IgE-mediated immune mechanisms involve Th2 delayed type hypersensitivity, and these responses are now sometimes referred to as hypersensitivities type I/type IV. CD8⁺ T cells can also cause delayed-type hypersensitivity. This is well characterized, for example, in poison ivy contact dermatitis, in which a lipid soluble molecule in the plant, pentadecacatechol, causes modification of intracellular proteins which then are presented on MHC class I to CD8⁺ T cells and elicit a CD8⁺ T-cell response. Inter-retinal photoreceptor-binding protein-specific CD8⁺ T cells have recently been shown to cause EAU; and therefore one could say that EAU may consist of both CD4⁺ T-cell and CD8⁺ T-cell delayed type hypersensitivity (52).

SPECIFIC OCULAR MANIFESTATIONS OF IMMUNOLOGIC PROCESSES

Cornea

The uninflamed cornea does not possess lymphatic drainage or vascular access. Immune competent cells can gain access to the cornea only via the limbal circulation or the anterior chamber. For these reasons there is both diminished exposure to antigen and diminished ability of immune cells to access the cornea. APCs are resident in the cornea, in the form of occasional Langerhans cells seen in the corneal stroma, but these cells express MHC class II antigens only when the cornea is inflamed. This relative immune seclusion of the cornea explains, for example, the low rate of corneal allograft rejection and the reduced importance of HLA typing in corneal transplants as compared to solid organ transplants. Conversely, the allograft rejection rate in vascularized corneas is much higher. Important cell-mediated

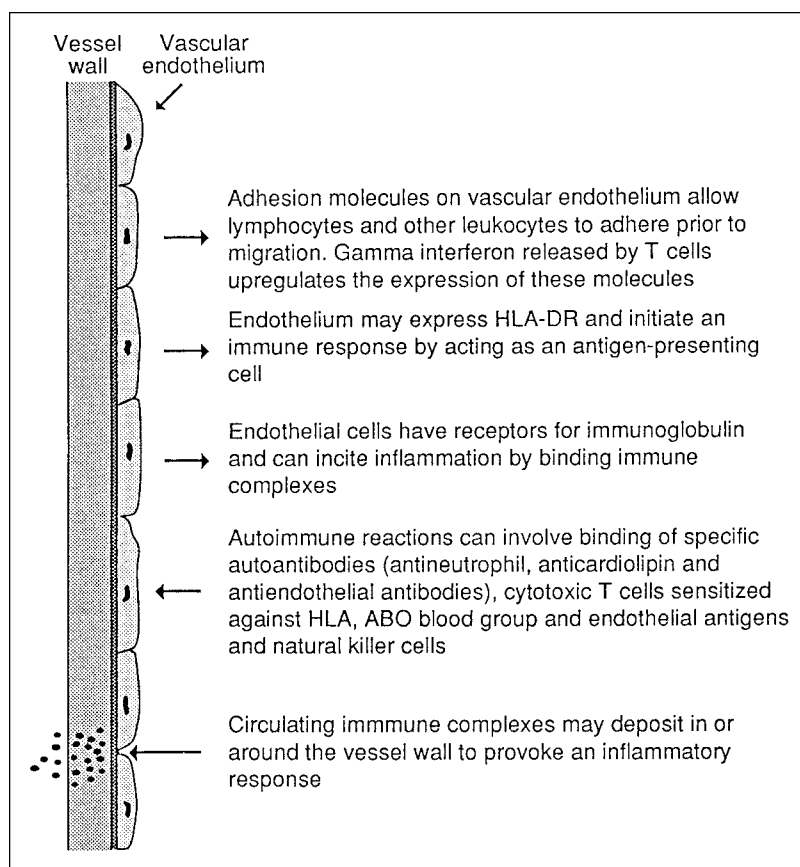


Figure 10 Mechanism of immunologically mediated vasculitis. *Source:* Courtesy of Dr. Amjad H.S. Rahi.

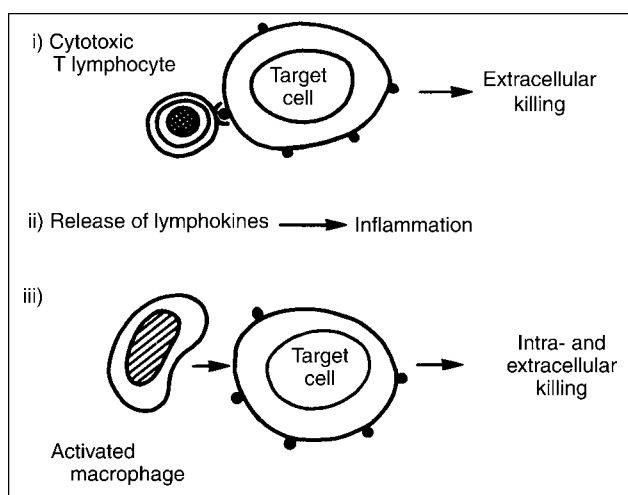


Figure 11 Cell-mediated hypersensitivity type IV: (i) cytotoxic CD8⁺ T cells kill target cells by inserting membrane-bound perforin (similar to the C9 fragment of complement) and inducing a permeability defect; (ii) release of cytokines (lymphokines) by the Th1 subset of helper T cells promotes a chronic inflammatory reaction; (iii) macrophages activated by interferon- γ as well as natural killer and lymphokine-activated cells, are also implicated. *Source:* Courtesy of Dr. Amjad H.S. Rahi.

immune reactions occur in the cornea. Perhaps best studied is the immune response directed toward herpes simplex virus (HSV) in the corneal stroma, in herpetic stromal keratitis (see Chapter 8) (58). Some but not all strains of mice in which corneas are infected with HSV-1 will develop herpetic stromal keratitis (HSK). Athymic nude mice that lack mature T lymphocytes will not develop HSK. However, if exogenous anti-HSV T cells are introduced by adoptive transfer the mice will develop HSK. Antibodies diffuse easily through the cornea, and antibody-antigen rings can be readily seen in experimental situations. Such reactions may also be involved in peripheral corneal changes seen in rheumatoid arthritis and similar collagen vascular diseases.

Anterior Chamber

A great deal of research has gone into dissecting the mechanisms of immune privilege in the eye. Immune privileged sites are defined as places in the body where foreign tissue grafts enjoy extended or indefinite survival. An altered, inhibited immune response in immune privileged sites decreases collateral damage resulting from inflammation where it is in

the best interest of the organism. Such sites include the testis, brain, and eye. It has become clear that the immune response responsible for immune privilege is not completely inhibited, but is distinguished or deviated from the response seen at non-privileged sites. Hence the mechanism of immunity in the eye is termed immune deviation. While other parts of the eye may also exhibit deviated immune responses, such as the vitreous (57) and subretinal space (66), the anterior chamber has been the best studied.

Many factors are thought to contribute to the immune privilege of the anterior chamber (59): (i) absence of blood vessels in the cornea, (ii) specialized endothelium of the iris stroma, (iii) absence of lymphatic drainage of the anterior chamber, (iv) direct drainage of the anterior chamber fluid into the venous circulation, (v) soluble molecules in the anterior chamber fluid, (vi) surface molecules on surrounding cells and (vii) tolerance-promoting APCs in the iris stroma and trabecular meshwork. Normal murine anterior chamber fluid has been shown to suppress CD4⁺ T-cell proliferation, PMN activation, macrophage activation, and C1q (a subcomponent of complement 1) and complement C (C3). TGF β is present in the aqueous humor, and probably contributes to partial inhibition of the immune response. Corneal endothelial cells have been shown to express CD46, CD55 and CD59 (Table 1) known to inhibit complement activation, as well as FAS ligand, which has been found to promote T-cell apoptosis. Consequently, allogenic tumor cells which are routinely rejected from conventional sites often progressively grow in the anterior chamber.

The interaction of cells introduced in the anterior chamber with the host's immune system results in what is termed an ACAID. Mice with growing allogenic tumors in the anterior chamber that are normally rejected in conventional transplant sites are unable to reject skin grafts genetically identical to the anterior chamber allogenic tumors. They fail to display delayed hypersensitivity to the tumors. They do, however, develop antibodies and cytotoxic T cells directed against the tumor. Even more surprising is that spleens of the anterior chamber tumor-bearing mice contain regulatory T cells that suppress donor-specific delayed hypersensitivity when adoptively transferred to naïve mice. In general, antigen introduced in the anterior chamber results in a deviated immune response with relatively inhibited CD4⁺ T cells (both Th1 cells and Th2 cells) and complement fixing antibody production, while supporting cytotoxic T cells and non-complement fixing antibody production. Not only is this immune deviation dependent on the host's spleen, but also the immune deviation is lost after splenectomy, if performed in the first 5 days after an introduction of antigen. ACAID

has been demonstrated in multiple animal models including mice, rats, rabbits and monkeys.

Conjunctiva

The conjunctiva is devoid of lymphocytes at birth and lymphocytes, predominantly B cells with some T cells, accumulate in the conjunctival stroma, forming extranodal conjunctiva-associated lymphoid tissue, termed CALT. These cells are largely responsible for IgM and IgE secretion. Toll-like receptors are recently discovered receptors on immune competent cells that recognize pathogen-associated molecular patterns and thereby initiate an antigen-nonspecific immune response. For example, Toll-like receptors can bind lipopolysaccharide in a manner independent of the T-cell receptor or antibody, and are fundamental in initiating innate and adaptive immunity. These receptors can lead to expression of a variety of pro-inflammatory cytokines and/or costimulatory molecules. Toll-like receptors have been localized to lymphocytes, eosinophils, mast cells and epithelial cells of the conjunctiva, and likely play a role in regulating local immune responses (5).

Lens

The lens is largely composed of crystalline proteins and albumin derivatives that are seen only in low concentrations elsewhere in the body, and consequently produce immunologic ignorance. These proteins are separated from immune surveillance by the intact lens capsule which is fully formed before the complete development of a competent immune system. Furthermore, the lens is shielded from the immune system partially by existing in an immune privileged site. Sudden exposure to higher concentrations of lens proteins has been shown to elicit an Arthus reaction and is likely to be the main mechanism of lens-induced uveitis (see Chapter 4).

Uvea

The choroid experiences an extremely high blood flow, so providing an efficient window to the circulatory system. The choroid normally contains few lymphocytes, but after inflammation begins it has the capacity to become extensively infiltrated by immune competent cells. It has long been postulated that uveitis can be caused by immune responses to ocular antigens. In the 1970s Wacker et al. (44,62,63) described a uveitogenic protein, termed S-antigen (currently designated arrestin), that could induce uveitis in guinea pigs if injected intradermally. Later, other uveitogenic ocular proteins were identified: interphotoreceptor retinoid-binding protein (IRBP), which functions as a transporter of retinoids between the retina and retinal pigment epithelium (RPE), rhodopsin, phoducin and recoverin, to name a few. These antigens are all present in the

retina and not in the choroid, implying that choroiditis is the site of inflammatory reactions directed against the overlying retina. T cells are found to be the predominant infiltrating cells in the early course of disease. SO and VKH have many clinical and histologic similarities to EAU. Marak et al. (27) found that peripheral blood lymphocytes from patients with SO proliferate when stimulated with RPE and retinal extract, but not choroidal extract. Choroid contains dendritic cells and these cells may play a role in antigen presentation and induction of uveitis. Such APCs have not been detected in the retina.

Retina

Although the uninflamed retina is relatively protected from the immune system by the blood-retinal barrier, it has been shown to interact with the immune system in retinal diseases. The retina has been shown to be the source of numerous antigens important in posterior segment inflammation, as indicated above. Among the retinal antigens that have been shown to be uveitogenic in animal models is recoverin, a calcium-binding protein found in the retina. This protein has been found to be a target of humoral immunity thought to be responsible for the paraneoplastic syndrome of cancer-associated retinopathy (see Chapter 27). Other retinal antigens, such as arrestin, IRBP, and rhodopsin, are considered to be responsible for the development of chorioretinitis in clinical disease, as discussed above (2). When these retinal antigens are injected with adjuvant into susceptible strains of various species, including guinea pigs, rabbits, rats, monkeys and mice, a predominantly CD4⁺ T-cell infiltrate of the retina, uvea, anterior chamber, vitreous cavity, optic nerve and pineal gland (since these proteins are also found in the pineal gland) develops. IRBP induces an accelerated more profound inflammation as compared to arrestin in most species, but a broad spectrum of severity can be elicited depending on the concentration of antigen, adjuvant, immunization schedule and species used. In contradistinction, non-retinal antigens such as melanin protein-associated insoluble antigens and the melanocyte differentiation antigen, tyrosinase family protein, produce experimental melanin-protein induced uveitis which tends to spare the retina. Ocular inflammation is mostly confined to the iris, ciliary body and choroid. Tyrosinase family protein models of uveitis may have special relevance to the study of VKH (see Chapter 4), since rats immunized with tyrosinase protein develop not only uveitis, but also vitiligo, and meningitis (69).

It is likely that these antigens are presented to lymphocytes at the initiation of the immune response locally in the retina. Potential APCs in the retina include vascular endothelial cells, RPE, Müller cells

and microglia, all of which have been shown to upregulate MHC class II molecules under certain experimental conditions. Nevertheless, in vivo antigen presentation by any of these cells has not yet been demonstrated.

The effector arm of the immune response in the retina has been well studied in EAU. The infiltration of effector cells to the retina, such as macrophages, and the activation and migration of resident cells, such as microglia, results in the release of proteolytic enzymes and reactive oxygen free radicals such as O₂⁻ and NO (67). Oxygen free radicals cause lipid peroxidation of the polysaturated fatty acid decosahexaenoic acid (22:6), which is a major component of the photoreceptor cell membrane. This causes direct damage to the photoreceptor and has been shown to serve as a chemotactic agent for PMNs. Interestingly, even prior to the deployment of the effector immune response in EAU, another oxygen free radical, peroxynitrite, is produced in photoreceptor mitochondria and results in nitration of at least three mitochondrial proteins including cytochrome c (68). This results in early photoreceptor damage in EAU, seemingly independent of effector lymphocyte, macrophage or microglial function. This reaction is presumably caused by the release of cytokines, such as TNF α , which is known to potentially upregulate nitric oxide synthetase by retinal or choroidal lymphocytes or mast cells in the choroid. The exact mechanisms leading to this early photoreceptor damage remains obscure.

Chronic inflammation plays a role in age-related macular degeneration (AMD) (see Chapter 18). The constituents of drusen include Ig, complement components, immune complexes, and several immunocompetent molecules, such as MHC class II molecules, vitronectin, clusterin, apolipoprotein E (ApoE) and complement receptor 1 (17,34). In the later stages of AMD, multinucleated giant cells and leukocytes have been identified in the choroid and in excised neovascular membranes. Patients with bilateral drusen or exudative AMD have a significantly higher serum level of antiretinal antibodies than age-matched controls (41). In animal models of choroidal neovascularization, macrophage depletion is associated with a reduced size, cellularity and vascularity of choroidal neovascular complexes (13). Although immune mechanisms participate in the development of AMD as described above, it is not clear that inflammation plays a causative role in AMD.

Lacrimal Gland

Large numbers of B cells and T cells are found in the lacrimal gland and account for antibody production and secretion into the tear film. IgA is the dominant antibody class, although IgG and IgM are also present.

A proportion of the IgG in tears is derived from the circulation and may target antigens encountered systemically. It has also been found that some of the locally produced IgA targets antigens presented at non-ocular sites. Increased lymphocytic infiltration of the lacrimal gland and subsequent atrophy of the lacrimal epithelium are seen in autoimmune causes of dry eye such as Sjögren syndrome (SS) (see Chapter 6) (31). A majority of these infiltrating lymphocytes are CD4⁺ T cells, resulting in a rise of the CD4⁺ T-cell/CD8⁺ T-cell ratio from 0.3 in normal lacrimal glands to 3.0 in SS (42). The cause of this infiltration is not clearly understood, but is thought to possibly involve viral infections in genetically predisposed individuals. It is noteworthy that the lymphocytic infiltration of the lacrimal gland increases with age. Inhibition of T-cell function, via cyclosporine, has proven useful in increasing tear production in animal models and in one clinical trial, resulting in topical cyclosporine therapy for dry eye (50). More specific immunomodulating targets have been recently explored as well. For example, adenovirus-mediated transfer of a TNF α inhibitor in a rabbit autoimmune dacryoadenitis resulted in increased tear production and tear stability, as well as a decrease in the CD4⁺ T-cell/CD8⁺ T-cell ratio (61).

IMMUNOLOGIC PROCESSES AND OCULAR INFECTIONS

All clinical infections represent the interactions of the pathogenic organism and the host immune system according to the mechanisms that have outlined above. Specific insights into the better studied ocular infections are provided below.

Viral Infection

Virus infections are discussed in Chapter 8 and immunologic processes play an important role in these diseases. Peptide derivatives of intracellular viral antigens are processed in the endoplasmic reticulum and presented to T lymphocytes on the host cell surface in the peptide groove of MHC class I molecules. CD8⁺ T lymphocytes with the appropriate CD3 for that specific peptide/MHC combination are then activated, undergo clonal expansion and elicit an adaptive, antigen specific cytotoxic response, destroying all cells displaying the same peptide. Similarly, extracellular viral particles are either phagocytosed, endocytosed in a receptor (IgG receptor)-mediated process, or pinocytosed. The antigens are then processed in the endosomal-lysosomal compartment of the cell, and expressed on the cell surface as peptides. When these cells interact with CD4⁺ T lymphocyte bearing the appropriate T-cell receptor, the T cell is activated, undergoes clonal expansion, and drives a Th1 or Th2 response. Th2 responses drive

class switching and lead to IgG, IgM and or IgE production. These antibodies can neutralize virus infectivity or lead to lysis of infected host cells by means of complement. Antibody binding to viral capsid or envelope prevents or causes conformational changes that preclude the virus' normal functions. Viruses that bud from the cell surface also bear viral surface antigens, to which antibody-mediated responses can be mounted. Viruses that are readily released and spread into the extracellular space, such as hepatitis and enteroviruses, are more susceptible to humoral immunity and patients with antibody deficiencies are more susceptible to these infections. In contrast viruses that tend to be more intracellular, such as herpes viruses or human immunodeficiency virus (HIV) are less susceptible to humoral immunity. Cellular responses to viruses include antigen-specific cytotoxic T-cell cytotoxicity, antibody-dependent cell-mediated cytotoxicity, NK cell cytotoxicity, and macrophage activation. Viruses that bind antibody can be cleared by different leukocytes that bear IgG receptors on their surface, including NK cells and macrophages. Additionally, NK cells can destroy virus-infected cells in an antibody-independent manner, by means of a variety of NK-cell receptors. Three types of IFN play important roles in viral infections. IFN α is secreted by lymphocytes and macrophages in response to viral and bacterial products, polynucleotides, tumor cells and allogenic cells. IFN β is produced by fibroblasts, epithelial cells, and leukocytes. T lymphocytes are capable of producing IFN γ when activated, promoting a Th1 response. When the IFNs interact with the infected cell, they induce intracellular protein production that inhibits translation of the viral genome. IFNs enhance T-cell cytotoxicity, enhance or depress antibody production, enhance macrophage phagocytosis, oxidative metabolism, and upregulate MHC class I and II expression. IFNs upregulate MHC class I expression on numerous cell types, and IFN γ upregulates MHC class II expression, even on cells that do not normally express it, such as RPE.

Viruses cause host damage by means of: (i) lysing host cells; (ii) transforming host cells; (iii) altering cell function; (iv) inducing inflammation; and (v) inciting autoimmune disease. An example of a virus-induced autoimmune disease is the immune destruction of myelin within the CNS following the experimental infection of the mouse with picornavirus (40). Similarly, Coxsackie viruses induce antibodies cross-reacting with myosin (molecular mimicry) that result in myositis (22).

Bacterial Infection

The conjunctiva and eyelid are colonized with numerous nonpathogenic bacteria as well as with bacteria capable of causing disease (see Chapter 9).

Many of these organisms produce disease because of immunologic hypersensitivities elicited in response to their antigens. For example, ribitol teichoic acid from *Staphylococcus* sp. may elicit antibody formation which forms immune complexes that are deposited typically at the corneoscleral limbus, where antibody and antigen concentrations and vascularity favor their deposition (33). These depositions can result in an infiltration of PMNs that leads to the formation of necrotic ulcers. The deposition of immune complexes and the subsequent lesions occur as an example of a hypersensitivity type III reaction.

Another immune-mediated manifestation of Staphylococcal blepharoconjunctivitis is the formation of phlyctenules, elevated limbal lesions that extend toward the central cornea with accompanying blood vessels (47). These represent a cell-mediated hypersensitivity type IV reaction, responding to Staphylococcal antigens. In the developing world this is more commonly seen with tuberculosis. These lesions typically occur in younger patients and run a self-limited 14-day course.

Chlamydia trachomatis, the causal agent of trachoma, causes a blinding chronic scarring follicular keratoconjunctivitis, afflicting over 500 million people worldwide (see Chapter 10). The disease is progressive, with the first exposure resulting typically in a mild self-limiting infection. Immunity is short-lived and following repeated exposure more severe inflammation and scarring develop. Conjunctival cultures during chronic disease often do not yield the infectious organism, suggesting that the chronic form may result from immune mechanisms. *Chlamydia psittaci* naturally infects guinea pigs producing an animal model of trachoma. In this model, repeated exposure to viable, but not replicating organisms results in clinical and histologic evidence of a hypersensitivity type IV (64). A prominent lymphocytic infiltrate accompanied by monocytes occurs in the conjunctiva not only after re-exposure of the conjunctiva to organism, but also after other non-ocular mucosal surfaces are inoculated. These findings strongly argue for the role of delayed type hypersensitivity in the pathogenesis of trachoma.

Fungal Infection

Fungal infections of the ocular tissues are discussed in Chapter 11. In some of these infections immunologic processes play an important role. For example, several components of the immune system and inflammatory response are important in ocular candidiasis. Several lines of evidence suggest that PMNs play a central role in the host response to *Candida* (14). The growth of *C. albicans* in vitro is inhibited by PMNs, granulocytopenic mice are highly susceptible to disseminated candidiasis, and stimulation of PMN function by

IFN γ is protective (14). Furthermore, TNF α knock-out animals exhibited increased mortality in response to experimental candidiasis, associated with a 10- to 1000-fold increase in yeast outgrowth in internal organs, delay in PMN recruitment, and a decreased ability to phagocytose *Candida* blastospores (36). Th1 responses are protective and are found to be important in the development of responses to reinfection, and important in driving phagocyte-dependent mucosal immunity (10). Antibodies have also been shown to be important in enhancing phagocytosis of *Candida* organisms (9). These studies indicate that immunity to *Candida* is complex and involves the interplay of multiple immune pathways; deficiency in one or more of these pathways results in increased susceptibility to infection. These experimental findings help explain, for example, why some patients without neutropenia develop chronic mucocutaneous candidiasis associated with decreased Th1 cytokine profiles (25).

PMNs are similarly essential in the host defense against *Aspergillus*. The clinical observation that *Aspergillus* endophthalmitis tends to occur only in the setting of granulocytopenia, while *Candida* endophthalmitis often occurs in the absence of granulocytopenia, suggests a greater role for PMNs in *Aspergillus* infection than in *Candida* infection (43). Tissue studies of eyes with *Aspergillus* endophthalmitis have revealed an almost exclusive growth of these organisms in the subretinal and the subretinal pigment epithelial spaces. This histopathologic finding is consistent with the finding that RPE contains numerous antioxidant proteins, such as superoxide dismutase, catalase, glutathione peroxidase, and other antioxidant enzymes, that inhibit the function of oxidants produced by PMNs. These antioxidant proteins likely hinder the PMN mediated clearance of *Aspergillus* by such oxidants as superoxide, hydrogen peroxide, and related metabolites.

Parasitic Infection

The interaction between parasites and the immune system is complex and parasites have evolved mechanisms so that they can survive the immunologic processes of the host that normally protect against foreign invaders (see Chapters 12 and 13). In cases where parasites are recognized by the immune system, a characteristic histopathologic finding of amorphous eosinophilic material surrounded by granulomatous inflammation with an abundance of eosinophils occurring in response to parasitic infections as well as other infectious and non-infectious processes has been termed the Splendore-Hoeppli phenomenon (46). This phenomenon has been observed in the conjunctiva; however, an organism has been found in only a minority of cases, suggesting that often the

inflammatory process is successful in eliminating the pathogenic parasite. An immunohistochemical analysis of the amorphous central eosinophilic material found in the conjunctiva has revealed predominantly complement proteins in some specimens and the major basic protein of eosinophils in other cases. Eosinophil major protein, one of four cationic proteins found in cytoplasmic granules of eosinophils, is toxic to helminths. A histopathologic study of two cases has suggested that the immune response and formation of eosinophilic granuloma consists first of antibody deposition, immune complex formation and complement fixation in the early stages, followed by recruitment and degranulation of eosinophils in later stages (46).

The protozoan obligate intracellular parasite *Toxoplasma gondii* commonly infects humans and is a leading cause of posterior uveitis (see Chapter 12). Infection results in IgM and IgG antibodies but these are insufficient to provide immunity because the organism persists intracellularly. As one would expect, the intracellular parasite elicits a predominantly CD8⁺ T-cell response. The T cells in turn release cytokines particularly IFN γ that activate macrophages, recruiting them to sites of infection where they function both as effector and APCs. *Toxoplasma* actively invades both phagocytic and nonphagocytic cells, forming a non-fusogenic compartment, called the parasitophorous vacuole, within the host cell cytosol (49). This compartment lacks many host cell membrane proteins and is unable to fuse with cell lysosomes. In contrast dead or opsonized parasites are taken up by phagocytes into normally functioning intracellular vacuoles which fuse with lysosomes thereby introducing the pathogens to an acidified hydrolytic environment. By blocking fusion with lysosomes the organism prevents pathogen-derived peptides from being expressed on the host cell's MHC molecules and evade the host immune system. Hence *Toxoplasma* may remain dormant within intracytoplasmic vesicles without exciting an immune response. Intracellular *Toxoplasma* has also been shown to actively interfere with transcription factors necessary for cytokine expression, including IL12, IFN γ , TNF α , and others (49). Furthermore, induced apoptotic pathways in macrophages infected with *Toxoplasma* are strongly inhibited, allowing intracellular parasites to survive longer in the host cell.

Once *Toxoplasma* replicates, the host cell becomes destroyed and the pathogen is opsonized and engulfed by macrophages which can then present antigen to both CD4⁺ T cells that typically produce Th1 cytokines and CD8⁺ T cells that eliminate infected cells and further drive the inflammatory process. Hypersensitivity reactions have also been implicated in toxoplasmosis. Results from monkey studies (65) have suggested that while retinochoroiditis results from an immune response directed

toward heat-inactivated organism, vitritis, vasculitis, anterior uveitis and neuritis may result from hypersensitivity reactions. The immune mechanisms of these reactions have not been well characterized.

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