CHAPTER 1

Basic Concepts in Immunology: Effector Cells and the Innate Immune Response

Highlights

- An immune response is the process for eliminating an offending stimulus. The clinical evidence of an immune response is inflammation.
- The immune system is composed of tissues, cells, and molecules that mediate response to infection or foreign material.
- Immune responses are broadly defined as innate or adaptive with close interaction between the two.
- Innate (natural) immunity provides immediate protection and requires no prior contact with the foreign substance or organism.
- Adaptive (acquired) immunity develops more slowly than innate immunity but provides more specific defense against infections.

Definitions

An immune response is a sequence of molecular and cellular events intended to rid the host of a threat: offending pathogenic organisms, toxic substances, cellular debris, or neoplastic cells. There are 2 broad categories of immune responses, *innate* and *adaptive*.

Innate immune responses, or *natural immunity,* require no prior contact with or "education" about the stimulus against which they are directed. Adaptive (or *acquired*) responses are higher-order, more specific responses directed against unique antigens. The term *antigen* refers to any substance (eg, toxin, foreign protein, bacterium) that can induce an immune response. Chapter 2 discusses adaptive responses in detail. This chapter introduces the crucial cells of the immune system and their functions in innate immunity.

Abbas AK, Lichtman AH, Pillai S. *Basic Immunology: Functions and Disorders of the Immune System.* 5th ed. Elsevier/Saunders; 2016.

Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology.* 9th ed. Elsevier/Saunders; 2018.

Murphy KM. *Janeway's Immunobiology.* 8th ed. Garland Science; 2012.

Cellular Components of the Immune System

White blood cells, or *leukocytes,* include several kinds of nucleated cells that can be distinguished by the shape of their nuclei and the presence or absence of cytoplasmic granules, as well as by their uptake of various histologic stains (Fig 1-1). They can be broadly divided into 2 subsets:

- myeloid (neutrophils, eosinophils, basophils and mast cells, monocytes and macrophages, and dendritic cells and Langerhans cells)
- lymphoid (T lymphocytes, B lymphocytes, and natural killer cells)

See BCSC Section 4, *Ophthalmic Pathology and Intraocular Tumors,* for additional discussion of leukocytes and findings on histologic examinations.

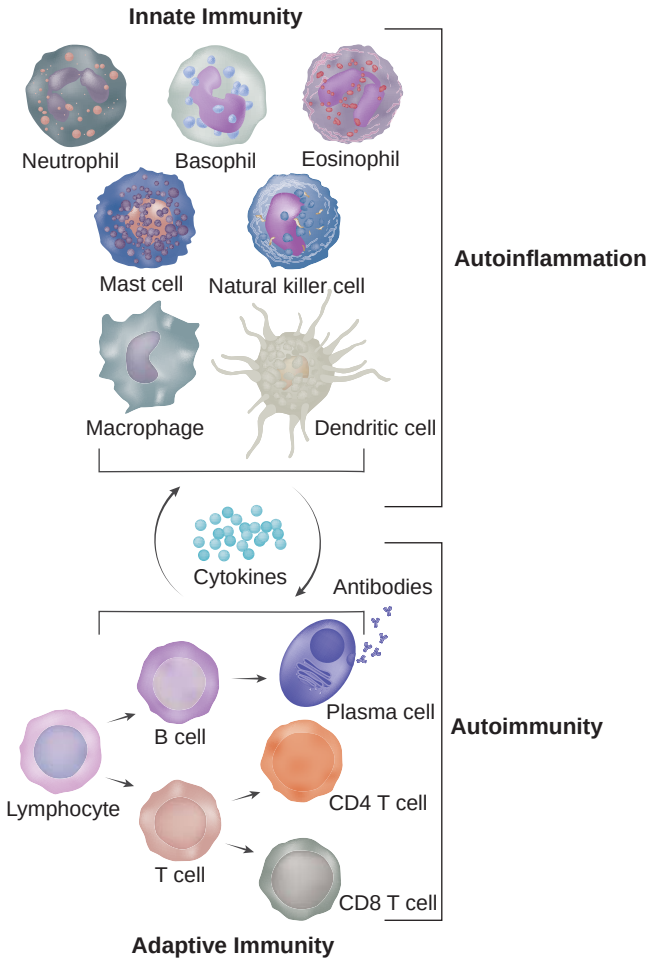

Figure 1-1 Cells of the immune system. *(Original illustration by Jared E. Knickelbein, MD, PhD. Redrawn by Cyndie C. H. Wooley.)*

Neutrophils

Neutrophils possess a multilobed nucleus of varying shapes; hence, they are also called *polymorphonuclear leukocytes* or *polymorphonuclear neutrophils (PMNs)*. Neutrophils also feature cytoplasmic granules and lysosomes and are the most abundant granulocytes in the blood. They are efficient phagocytes that readily clear tissues, degrade ingested material, and act as important effector cells through the release of granule products and cytokines.

During the beginning or acute phases of inflammation, neutrophils are one of the first inflammatory cells to migrate from the bloodstream toward the site of inflammation. This process is called *chemotaxis*. Neutrophils dominate the inflammatory infiltrate in experimental models and clinical examples of active bacterial infections of the conjunctiva (conjunctivitis), sclera (scleritis), cornea (keratitis), and vitreous (endophthalmitis). They are also dominant in many types of active viral infections of the cornea (eg, herpes simplex virus keratitis) and retina (eg, herpes simplex virus retinitis).

Eosinophils

Eosinophils are characterized by the presence of a bilobed nucleus and abundant lysosomes and cytoplasmic granules that consist of more basic protein than that found in other polymorphonuclear leukocytes. These basic proteins bind acidic dyes, such as eosin—hence the name eosinophil. Eosinophilic granule products, including major basic protein and ribonucleases, destroy parasites efficiently. Eosinophils accumulate at sites of parasitic infection. They are also important in allergic immune reactions. Eosinophilia in the peripheral blood may occur in both parasitic infections and allergic disease.

Eosinophils are abundant in the conjunctiva and tears in many forms of allergic conjunctivitis, especially atopic and vernal conjunctivitis. They are not considered major effectors for intraocular inflammation, with the notable exception of helminthic infections of the eye, especially toxocariasis.

Basophils and Mast Cells

Basophils are the blood-borne equivalent of the tissue-bound mast cell. There are 2 major types of mast cells, connective tissue and mucosal. Both can release preformed granules and synthesize certain mediators de novo that differ from those of neutrophils and eosinophils. *Connective tissue mast cells* have abundant granules containing histamine and heparin, and they synthesize prostaglandin D_2 upon stimulation. In contrast, *mucosal mast cells* normally contain low levels of histamine and require T-cell–derived growth-promoting cytokines for stimulation. Stimulated mucosal mast cells primarily synthesize leukotrienes, in particular leukotriene C_4. Tissue location can alter the granule type and functional activity, but regulation of these differences is not well understood.

Mast cells act as major effector cells in immunoglobulin (Ig) E–mediated inflammatory reactions, especially of the allergic or immediate hypersensitivity type. They perform this function through their expression of high-affinity Fc receptors for IgE. *Fc* (from "*f*ragment, *c*rystallizable") refers to the constant region of immunoglobulin that binds cell surface receptors (see Chapter 2). Mast cells may also participate in the induction of cell-mediated

immunity, wound healing, and other functions not directly related to IgE-mediated degranulation. Other stimuli, such as complement or certain cytokines, may also trigger degranulation.

The healthy human conjunctiva contains numerous mast cells localized in the substantia propria. In certain atopic and allergic disease states, such as vernal conjunctivitis, the number of mast cells increases in the substantia propria and the epithelium, which is usually devoid of mast cells. The uveal tract also contains numerous connective tissue mast cells, whereas the cornea has none.

Monocytes and Macrophages

Monocytes, the circulating cells, and macrophages, the tissue-infiltrating equivalents, are important effectors in innate and adaptive immunity. These mononuclear cells are often detectable in acute ocular infections, even if other cell types, such as neutrophils, are more numerous. Monocytes are relatively large cells (12–20 μm in suspension and up to 40 μm in tissues) that normally travel throughout the body. Most tissues have at least 2 identifiable macrophage populations: tissue resident and blood derived. Although exceptions exist, tissue-resident macrophages are monocytes that migrated into tissue during embryologic development and later acquired tissue-specific properties and cellular markers. Various resident macrophages have tissue-specific names (ie, Kupffer cells in the liver, alveolar macrophages in the lung, and microglia in the brain and retina). Blood-derived macrophages are monocytes that have recently migrated from the blood into a fully developed tissue site.

Macrophages may serve in 3 capacities:

- sentinels that recognize danger signals from pathogens and/or tissue damage
- effectors that induce inflammation and fight pathogens directly
- regulatory/repair cells that conduct tissue repair, regulate the adaptive immune system, and serve as checkpoints during immune cell migration

Various signals can prime resting (immature or quiescent) monocytes for differentiation into efficient antigen-presenting cells (APCs). Upon additional signals, these APCs are activated to become effector cells. Effective activation stimuli include exposure to bacterial products, such as lipopolysaccharide; phagocytosis of antibody-coated or complement-coated pathogens; or exposure to mediators released during inflammation, such as interleukin (IL) 1β or interferon gamma.

Only after full activation do macrophages become most efficient at the synthesis and release of inflammatory mediators and the killing and degradation of phagocytosed pathogens. Activated macrophages may terminally differentiate into epithelioid cells, with larger nuclei, abundant cytoplasm, and indistinct cell borders, resembling squamous epithelium. These epithelioid histiocytes are characteristic of granulomatous inflammation, either in infectious uveitis (ie, tuberculosis, syphilis, herpes, fungal infection, parasitic uveitis) or noninfectious uveitis (ie, sarcoidosis, granulomatosis with polyangiitis). Activated macrophages may also fuse to form *multinucleated giant cells,* which may accompany granulomatous inflammation or occur in the tissue reaction to foreign material.

Dendritic Cells and Langerhans Cells

Dendritic cells (DCs) are terminally differentiated, bone marrow–derived mononuclear cells that are distinct from macrophages and monocytes. These specialized cells bridge the innate and adaptive immune systems but do not directly participate in effector activities. DCs use pattern recognition receptors, such as Toll-like receptors, to recognize pathogens. Activated DCs upregulate costimulatory molecules and produce cytokines to drive T-cell priming and effector differentiation as well as activate various types of immune cells. Interestingly, antigen presentation by nonactivated, steady-state DCs might lead to T-cell unresponsiveness, promoting tolerance.

All human DCs express high levels of human leukocyte antigen (HLA) class II molecules and may be classified by lineage markers as myeloid/classical or plasmacytoid. DCs can also be classified functionally and anatomically, as their function is linked to their location:

- Blood DCs are precursors of tissue and lymphoid organ DCs.
- Migratory or tissue DCs reside in most epithelial tissues, where they acquire antigen and from which they migrate via afferent lymphatics to lymph nodes. In tissue sites, DCs are large (15–30 μm), with cytoplasmic veils that form extensions 2–3 times the diameter of the cell and resemble the dendritic structure of neurons.
- Resident or lymphoid DCs arise in lymph nodes directly from the blood.
- Inflammatory DCs are present in tissues and lymphoid organs during inflammation. Precursors include classical monocytes.

Langerhans cells (LCs) are myeloid cells with DC function that reside in the epidermis and stratified epithelia of the cornea, as well as conjunctival, buccal, gingival, and genital mucosae. LCs are identified by their many dendrites, electron-dense cytoplasm, and Birbeck granules. Interestingly, they originate from primitive hematopoiesis in the yolk sac and form a stable, self-renewing network that does not require bone marrow–derived precursors in the absence of inflammation. At rest, they are not active APCs. On activation, LCs lose their granules and transform to resemble blood and lymphoid DCs. Evidence suggests that LCs migrate along the afferent lymph vessels to the draining lymphoid organs.

LCs are important components of the immune system and play roles in antigen presentation, control of lymphoid cell traffic, differentiation of T lymphocytes, and induction of delayed hypersensitivity. Elimination of LCs from skin before an antigen challenge inhibits induction of the contact hypersensitivity response. In the conjunctiva and limbus, LCs are the only cells that constitutively express HLA class II molecules. LCs are present in the peripheral cornea, and any stimulation of the central cornea results in central migration of the peripheral LCs.

Lymphocytes

Lymphocytes are small (10–20-μm) cells with large, round, and dense nuclei. Like DCs, they are derived from stem cell precursors within the bone marrow; however, unlike other leukocytes, lymphocytes require subsequent maturation in peripheral lymphoid organs.

Three broad categories of lymphocytes are

- T lymphocytes (also called *T cells*)
- B lymphocytes (also called *B cells*)
- non-T, non-B lymphocytes

The expression of specific cell surface proteins (ie, *surface markers*) can be used to further divide lymphocytes. These markers are in turn related to the functional and molecular activity of individual subsets. Two types of lymphocytes participate in the innate immune response, serving as a bridge between innate and adaptive responses: (1) gamma-delta (γδ) T cells or sentinel T cells, also known as *intraepithelial lymphocytes;* and (2) natural killer (NK) cells, a subset of non-T, non-B lymphocytes. Chapter 2 discusses the roles of lymphocytes in adaptive immunity.

Overview of the Innate Immune System

The innate immune system is a relatively broad-acting rapid reaction force that recognizes nonself (foreign) substances, proteins, or lipopolysaccharides. The innate response is immediate and requires no prior exposure to the foreign substance. Innate immune responses are driven by germline-encoded receptors that recognize features common to many pathogens and offending stimuli. The result is the generation of biochemical mediators and cytokines that recruit innate effector cells, especially macrophages and neutrophils, to remove the offending stimulus through phagocytosis or enzymatic degradation. The innate response also alerts the cells of the adaptive immune system to reinforce and refine the attack. For example, in acute endophthalmitis, bacteria-derived toxins or host cell debris stimulates the recruitment of neutrophils and monocytes, leading to the production of inflammatory mediators and phagocytosis of the bacteria.

An array of highly conserved pattern recognition receptors (PRRs) and proteins detect similarly conserved molecular motifs—*pathogen-associated molecular patterns (PAMPs)*—on triggering stimuli. PAMPs include proteins found in bacterial, fungal, and viral nucleic acids. Cell-associated PRRs may be extracellular, endosomal, or cytoplasmic and include Toll-like receptors (TLRs), C-type lectin receptors (CTLRs), nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, or NLRs), and retinoic acid–inducible gene-I-like receptors (RIG-I-like receptors, or RLRs). Since each PRR has evolved to respond to a different PAMP, engagement of a specific PRR subtype conveys information about the type of infection (ie, bacterial, fungal, or viral) and the location (ie, extracellular or intracellular). In humans, there are 10 members of the TLR family. Those that respond to bacterial products (TLR1/2, TLR2/6, TLR4, TLR5) are localized in the plasma membrane of innate immune cells and sense extracellular microbes. The TLRs that detect viral nucleic acids (TLR3, TLR7, TLR9) are located within endosomal compartments and interact with membrane proteins.

Immunity Versus Inflammation

An immune response is the process for removing an offending stimulus. An immune response that becomes clinically evident is termed an *inflammatory response* (see Clinical

CLINICAL EXAMPLE 1-1

Autoinflammatory and Autoimmune Diseases

Dysregulation and overactivity of either innate or adaptive immune responses can lead to clinically evident inflammatory disease. Autoimmune disease results from loss of adaptive immune tolerance to autoantigens (also called *self antigens*) that leads to a dysregulated immune attack on self tissues. Autoreactive B and T cells of the adaptive immune system (discussed further in Chapter 2) are critical to the disease process (see Fig 1-1). An example of a classic autoimmune disease is systemic lupus erythematosus. More recently, the term *autoinflammatory disease* has been used to describe inflammatory conditions driven predominantly by overactivity of the innate immune system in the absence of pathologic B- or T-cell responses. An example of an autoinflammatory disease is juvenile idiopathic arthritis. Most inflammatory diseases likely lie on a spectrum between autoinflammation and autoimmunity. An example of a mixed-spectrum disease is ankylosing spondylitis. The etiology of inflammatory diseases is thought to be multifactorial, with genetic, environmental, and possibly infectious triggers.

Delves PJ, Martin SJ, Burton DR, Roitt IM. *Roitt's Essential Immunology.* 13th ed. Wiley-Blackwell; 2017.

Szekanecz Z, McInnes IB, Schett G, Szamosi S, Benkő S, Szűcs G. Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases. *Nat Rev Rheumatol.* 2021;17(10):585–595.

Example 1-1). Inflammatory responses typically result in 5 cardinal clinical manifestations: pain, hyperemia, edema, heat, and loss of function. These manifestations are the consequence of 2 physiologic changes within a tissue: cellular recruitment and altered vascular permeability.

The following pathologic findings are typical in inflammation:

- infiltration of effector cells resulting in the release of biochemical and molecular mediators of inflammation, such as cytokines (eg, interleukins and chemokines) and lipid mediators (eg, prostaglandins, leukotrienes, and platelet-activating factors)
- production of oxygen metabolites (eg, superoxide and nitrogen radicals)
- release of granule products as well as catalytic enzymes (eg, proteases, collagenases, and elastases)
- activation of plasma-derived enzyme systems (eg, complement components and fibrin)

These effector processes are described in greater detail later in this chapter.

Immune responses are a constant presence, though usually at a subclinical level. For example, ocular surface allergen exposure, which occurs daily, or the nearly ubiquitous event of bacterial contamination during cataract surgery is usually cleared by immune mechanisms *without* overt inflammation.

Innate Immunity: Triggers and Mechanisms

A variety of triggers and mechanisms are involved in innate immune responses of the eye. Four of the most important triggers are

- bacteria-derived molecules
- damage to nonimmune ocular parenchymal cells by toxins or trauma
- innate mechanisms for the recruitment and activation of neutrophils through the activation of vascular endothelial cells
- innate mechanisms for the recruitment and activation of macrophages

These are discussed in the following sections. Table 1-1 summarizes triggering molecules of innate ocular immune responses.

Bacteria-Derived Molecules That Trigger Innate Immune Responses

Bacterial lipopolysaccharide

Lipopolysaccharide (LPS), also known as *endotoxin,* is an intrinsic component of the cell walls of most gram-negative bacteria. Among the most important triggering molecules of innate immune responses, LPS consists of 3 components: lipid A, O polysaccharide, and core oligosaccharide. Lipid A is the most potent component, capable of activating effector cells at concentrations of a few picograms per milliliter. The exact structures of each component vary among species of bacteria, but all are recognized by the innate immune system. The primary receptors are the TLRs, principally TLR2 and TLR4, which are expressed on macrophages, neutrophils, and DCs, as well as on B cells and T cells.

The effects of LPS include the following:

- activation of monocytes and neutrophils, leading to upregulation of genes for various cytokines (IL-1, IL-6, tumor necrosis factor [TNF])
- degranulation
- activation of complement via the alternative pathway
- activation of vascular endothelial cells

Humans are intermittently exposed to low levels of LPS that the gut releases, especially during episodes of diarrhea and dysentery. Exposure to LPS may play a role in

Table 1-1 Triggering Molecules of Innate Immune Responses in the Eye

Bacteria-derived molecules
Lipopolysaccharide
Other cell wall components
Exotoxins and secreted toxins
Nonspecific soluble molecules (also modulate innate immunity)
Plasma-derived enzymes
Acute-phase reactants
Cytokines produced by parenchymal cells within a tissue site

dysentery-related uveitis, arthritis, and reactive arthritis. LPS is the major cause of shock, fever, and other pathophysiologic responses to bacterial sepsis, making it an important cause of morbidity and mortality during gram-negative bacterial infections. Interestingly, footpad injection of LPS in rodents results in an acute anterior uveitis. This animal model is called *endotoxin-induced uveitis*. See Clinical Example 1-2.

Other bacterial cell wall components

The bacterial cell wall and membrane are complex. They contain numerous polysaccharide, lipid, and protein structures that can initiate an innate immune response independent of adaptive immunity. Killed lysates of many types of gram-positive bacteria or mycobacteria can directly activate macrophages, making them useful as adjuvants. Some of these components have been implicated in various models for arthritis and uveitis. In many cases, the molecular mechanisms might be similar to those of LPS.

CLINICAL EXAMPLE 1-2

Role of Bacterial Toxin Production in the Severity of Endophthalmitis

In experimental models, intraocular injection of lipopolysaccharide (LPS) is highly inflammatory and accounts for much of the enhanced pathogenicity of gram-negative infections of the eye. For example, intravitreal injection of LPS triggers a dose-dependent neutrophilic and monocytic infiltration of the uveal tract, retina, and vitreous. Toll-like receptor 2 (TLR2) recognizes LPS, and binding of LPS by TLR2 on macrophages results in macrophage activation and secretion of a wide array of inflammatory cytokines, including interleukin (IL) 1, IL-6, and tumor necrosis factor α (TNF-α). Degranulation of platelets is among the first histologic changes in LPS-induced uveitis; likely mediators are eicosanoids, platelet-activating factors, and vasoactive amines. The subsequent intraocular generation of several mediators, especially leukotriene B_4, thromboxane B_2, prostaglandin E_2, and IL-6, correlates with the development of the cellular infiltrate and vascular leakage.

Using clinical isolates or bacteria genetically altered to diminish production of the various types of bacterial toxins, investigators have demonstrated that toxin elaboration in gram-positive or gram-negative endophthalmitis greatly influences inflammatory cell infiltration and retinal cytotoxicity. This effect suggests that sterilization through antibiotic therapy alone, in the absence of antitoxin therapy or toxin removal, may not prevent activation of innate immunity, ocular inflammation, and vision loss in eyes infected by toxin-producing strains.

Booth MC, Atkuri RV, Gilmore MS. Toxin production contributes to severity of *Staphylococcus aureus* endophthalmitis. In: Nussenblatt RB, Whitcup SM, Caspi RR, Gery I, eds. *Advances in Ocular Immunology: Proceedings of the 6th International Symposium on the Immunology and Immunopathology of the Eye.* Elsevier; 1994:269–272.

Exotoxins and other secretory products of bacteria

Certain bacteria secrete products known as *exotoxins* into their surrounding microenvironment. Many of these products are enzymes that, although not directly inflammatory, can cause tissue damage and subsequent inflammation and tissue destruction. Examples of these products include

- collagenases
- hemolysins such as streptolysin O, which can kill neutrophils by causing cytoplasmic and extracellular release of their granules
- phospholipases such as the *Clostridium perfringens* α-toxins, which kill cells and cause necrosis by disrupting cell membranes

An intravitreal injection of a purified hemolysin BL toxin derived from *Bacillus cereus* can cause direct necrosis of retinal cells and retinal detachment. In animal studies, as few as 100 *B cereus* organisms can produce enough toxin to cause complete loss of retinal function in 12 hours. In addition to being directly toxic, bacterial exotoxins can be strong triggers of an innate immune response.

Callegan MC, Jett BD, Hancock LE, Gilmore MS. Role of hemolysin BL in the pathogenesis of extraintestinal *Bacillus cereus* infection assessed in an endophthalmitis model. *Infect Immun.* 1999;67(7):3357–3366.

Murphy K, Travers P, Walport M. *Janeway's Immunobiology.* 8th ed. Garland Science; 2012.

Other Triggers or Modulators of Innate Immune Responses

Damage to nonimmune ocular parenchymal cells—especially iris or ciliary body epithelium, retinal pigment epithelium, retinal Müller cells, or corneal or conjunctival epithelium—by toxins or trauma can trigger innate immune responses in the eye. This damage can result in the synthesis of a wide range of mediators, cytokines, and eicosanoids. For example, phagocytosis of staphylococci by corneal epithelium, microtrauma to the ocular surface epithelium by contact lenses, chafing of iris or ciliary epithelium by an intraocular lens, or laser treatment of the retina can stimulate ocular cells to produce mediators that assist in the recruitment of innate effector cells such as neutrophils or macrophages. See Clinical Example 1-3.

CLINICAL EXAMPLE 1-3

Uveitis-Glaucoma-Hyphema Syndrome

One cause of inflammation following cataract surgery, uveitis-glaucoma-hyphema (UGH) syndrome, is related to the physical presence of certain types of intraocular lenses (IOLs). Although UGH syndrome was more common when rigid anterior chamber lenses were used during the early 1980s, it has also been reported with posterior chamber lenses, particularly when a haptic of a 1-piece lens is inadvertently placed in the sulcus. The pathogenesis of UGH syndrome appears related to mechanisms of innate immunity activation. A likely mechanism is cytokine and eicosanoid synthesis triggered by mechanical chafing of or trauma to the iris or ciliary

> body. Plasma-derived enzymes, especially complement or fibrin, can enter the eye through vascular permeability altered by surgery or trauma and can then be activated by contact with the surface of IOLs. Adherence of bacteria and leukocytes to the surface has also been implicated. Toxicity caused by contaminants on the lens surface during manufacturing is rare. Nevertheless, noninflamed eyes with IOLs can demonstrate histologic evidence of low-grade, foreign-body reactions around the haptics.

Innate Mechanisms for the Recruitment and Activation of Neutrophils

A key part of the innate immune response is the recruitment and activation of neutrophils, which are highly efficient effectors of this response. Neutrophils are categorized as *resting* or *activated,* according to their secretory and cell membrane activity. In the innate immune response, recruitment of resting, circulating neutrophils occurs rapidly in a tightly controlled process consisting of 2 events:

- neutrophil adhesion to the vascular endothelium through cell adhesion molecules (CAMs) on leukocytes as well as on endothelial cells, primarily in postcapillary venules
- transmigration of the neutrophils through the endothelium and its extracellular matrix, mediated by chemotactic factors

Activation of vascular endothelial cells is triggered by various innate immune stimuli, such as LPS, physical injury, thrombin, histamine, or leukotriene release. *Neutrophil rolling*—a process by which neutrophils bind loosely and reversibly to nonactivated endothelial cells—involves molecules from at least 3 sets of CAM families:

- *selectins,* especially E-, L-, and P-selectin
- *integrins,* especially leukocyte function–associated antigen 1 and macrophage-1 antigen
- *immunoglobulin superfamily molecules,* especially intercellular adhesion molecule (ICAM) 1 and ICAM-2

These molecules are expressed on both neutrophils and vascular endothelial cells.

The primary events are mediated largely by members of the selectin family and occur within minutes of stimulation (Fig 1-2). Nonactivated neutrophils express L-selectin, which mediates a weak bond to endothelial cells by binding to specific selectin ligands. Upon exposure to triggering molecules such as LPS, endothelial cells become activated, expressing in turn at least 2 other selectins (E and P) by which they can bind to the neutrophils and help stabilize the interaction in a process called *adhesion.* Subsequently, other factors, such as platelet-activating factor (PAF), various cytokines, and bacterial products can induce upregulation of the β-integrin family. As integrins are expressed, the selectins are shed, and neutrophils then bind firmly to endothelial cells through the immunoglobulin superfamily molecules.

After adhesion, various chemotactic factors are required in order to induce *transmigration* of neutrophils across the endothelial barrier and into the extracellular matrix of

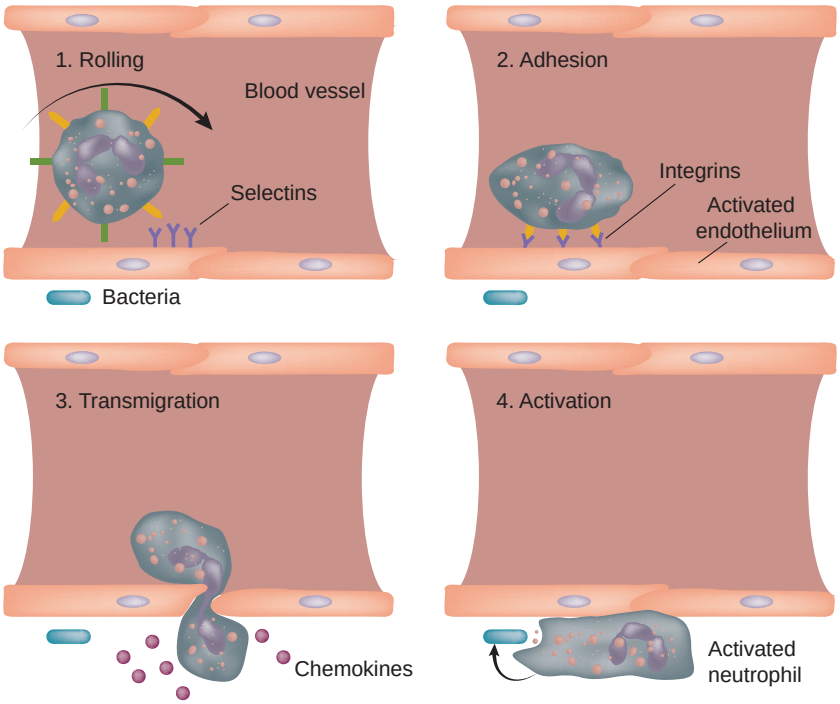

Figure 1-2 Steps of neutrophil migration and activation in infection. **1,** In response to innate immune stimuli, *rolling* neutrophils within the blood vessel bind loosely and reversibly to nonactivated endothelial cells by selectins. **2,** Neutrophil adhesion is mediated by E- and P-selectins, β-integrins, and immunoglobulin superfamily molecules, which are expressed on activated vascular endothelial cells in response to innate activating factors and bacterial products. **3,** Chemotactic factors triggered by the infection induce *transmigration* of neutrophils across the endothelial barrier into the extracellular matrix of the tissue. **4,** Finally, upon stimulation by bacterial toxins and phagocytosis, neutrophils are fully activated, becoming functional effector cells. *(Original illustration by Barb Cousins, modified by Joyce Zavarro; redrawn by Cyndie C. H. Wooley.)*

the tissue. Chemotactic factors are short-range signaling molecules that diffuse in a declining concentration gradient from the source of production within a tissue to the vessel. Neutrophils have receptors for these molecules and are induced to undergo membrane changes that cause migration in the direction of highest concentration. Examples of chemotactic factors include

- complement products, such as the anaphylatoxin C5a
- fibrin split products
- certain neuropeptides, such as substance P
- bacteria-derived formyl tripeptides, such as *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)
- leukotrienes
- α-chemokines, such as IL-8

Activation of neutrophils into functional effector cells begins during adhesion and transmigration but is fully achieved upon neutrophil interaction with specific signals within the site of injury or infection. The most effective activation triggers are bacteria

and their toxins, especially LPS. Other innate or adaptive mechanisms (especially complement) and chemical mediators (such as leukotrienes and PAF) also contribute to neutrophil activation. Neutrophils, unlike monocytes or lymphocytes, do not leave a tissue to recirculate but remain and die.

Phagocytosis

Phagocytosis of bacteria and other pathogens is a process mediated by receptors. The 2 most important are *antibody Fc receptors* and *complement receptors*. Pathogens in an immune complex with antibody or activated complement components bind to cell surface membrane–expressed Fc or complement receptors.

The area of membrane bound by the pathogen invaginates and becomes a phagosome. Cytoplasmic granules and lysosomes then fuse with the phagosomes. Phagocytes have multiple means of destroying microorganisms, notably, antimicrobial polypeptides residing within the cytoplasmic granules as well as reactive oxygen and nitrogen radicals. Although these mechanisms primarily destroy pathogens, released contents, such as lysosomal enzymes, may contribute to the amplification of inflammation and tissue damage.

Neutrophil-derived granule products

As mentioned earlier, neutrophils are proficient phagocytes that act as effector cells through the release of granule products and cytokines. Many antimicrobial polypeptides are present in neutrophilic granules. The principal ones are bactericidal/permeability-increasing protein, defensins, lysozyme, lactoferrin, and the serine proteases.

In addition to antimicrobial polypeptides, neutrophils contain numerous other molecules that contribute to inflammation. These compounds include hydrolytic enzymes, elastase, matrix metalloproteinases (MMPs), gelatinase, myeloperoxidase, vitamin B_{12}–binding protein, and cytochrome b_{558}. Granule contents remain inert and membrane bound when the granules are intact and become active and soluble when granules fuse to the phagocytic vesicles or plasma membrane. Collagenase is an example of an MMP found within neutrophilic granules. Various forms of collagenase contribute to corneal injury and liquefaction during bacterial keratitis and scleritis, especially in infections with *Pseudomonas* species. Collagenases also contribute to peripheral corneal melting syndromes secondary to rheumatoid arthritis–associated peripheral keratitis.

Innate Mechanisms for the Recruitment and Activation of Macrophages

Monocyte-derived macrophages are another important type of effector cell for the innate immune response that follows trauma or acute infection. The various molecules involved in monocyte adhesion and transmigration from blood into tissues are probably similar to those for neutrophils, although they have not been studied as thoroughly. The functional activation of macrophages, however, is more complex than that of neutrophils. Macrophages exist in different levels or stages of metabolic and functional activity, each representing different "programs" of gene activation and synthesis of macrophage-derived cytokines and mediators. The categories of macrophages include

- resting (immature or quiescent)
- primed

- activated
- stimulated or reparative (partially activated)

Resting and scavenging macrophages

Phagocytosis removes host cell debris in the process called *scavenging*. Resting macrophages are the classic scavenging cell, capable of phagocytosis and uptake of the following:

- dead cell membranes
- chemically modified extracellular protein (ie, acetylated or oxidized lipoproteins)
- sugar ligands, through mannose receptors
- naked nucleic acids
- bacterial pathogens

Resting monocytes express at least 3 types of scavenging receptors but synthesize very low levels of proinflammatory cytokines. Scavenging can occur in the absence of inflammation. See Clinical Example 1-4.

Primed macrophages

Resting macrophages become primed by exposure to certain cytokines. Upon priming, these cells become positive for HLA class II molecules and capable of functioning as APCs to T lymphocytes (see Chapter 2). Priming involves the following:

- activation of specialized lysosomal enzymes, such as cathepsins D and E, for degrading proteins into peptide fragments
- upregulation of specific genes (ie, major histocompatibility complex class II) and costimulatory molecules (ie, B7.1)
- increased cycling of proteins between endosomes and the cell surface membrane

CLINICAL EXAMPLE 1-4

Phacolytic Uveitis

Mild infiltration of scavenging macrophages centered around retained lens cortex or nucleus fragments occurs in nearly all eyes with lens injury, including those that have undergone routine cataract surgery. This infiltrate is notable for the *absence* of both prominent neutrophil infiltration and substantial nongranulomatous inflammation. An occasional giant cell may be present, but granulomatous changes are not extensive.

Phacolytic uveitis (phacolytic glaucoma) is a variant of scavenging macrophage infiltration in which leakage of lens protein occurs through the intact capsule of a mature or hypermature cataract. Lens protein–engorged scavenging macrophages block the trabecular meshwork outflow channels, resulting in elevated intraocular pressure. Other signs of typical lens-associated uveitis are conspicuously absent. Experimental studies suggest that lens proteins may be chemotactic stimuli for monocytes. See Chapter 8 for further discussion of the clinical presentation of phacolytic uveitis.

Primed macrophages thus resemble DCs. They can exit tissue sites by afferent lymphatic vessels to reenter the lymph node.

Activated and stimulated macrophages

Activated macrophages are classically defined as macrophages that produce the full spectrum of inflammatory and cytotoxic cytokines; thus, they mediate and amplify acute inflammation, tumor killing, and major antibacterial activity. *Epithelioid cells* and *giant cells* represent different terminal differentiations of the activated macrophage.

Activation of macrophages can occur through exposure to innate stimuli or various substances, such as the following:

- cytokines, including chemokines, derived from T lymphocytes and other cell types
- bacterial cell walls or toxins from gram-positive or acid-fast organisms
- complement activated through the alternative pathway
- foreign bodies composed of potentially toxic substances, such as talc or beryllium
- exposure to certain surfaces, such as some plastics

Activation of macrophages is also termed *polarization*. Macrophages can be classified as M1 or M2 based on the observation that certain stimuli produce distinct patterns of gene or protein expression under experimental conditions. For example, exposure to bacterial LPS and interferon gamma typically polarizes macrophages to the M1 phenotype, whereas activation by other innate stimuli in the absence of interferon gamma polarizes toward the M2 phenotype. The activation state seems to be somewhat reversible, suggesting that macrophages may switch between subsets (plasticity), depending on environmental signals. In fact, macrophage research suggests there are at least 9 distinct classes of macrophage activation. Thus, although the M1/M2 model may be oversimplified, it does provide a framework for conceptualizing different levels of macrophage activation in terms of acute inflammation (Fig 1-3).

M1–classically activated macrophage characteristics include

- high production of proinflammatory cytokines (eg, IL-1, IL-6, and TNF-α) and oxygen radicals
- expression of inducible nitric oxide synthase with production of reactive nitrogen intermediates
- promotion of T helper-1 (Th1) response
- strong microbicidal and tumoricidal activity

M2–alternatively activated macrophage characteristics include

- parasite containment
- promotion of tissue remodeling
- promotion of tumor progression
- immunoregulatory functions (eg, arginase and IL-10 secretion)

As noted, macrophages that are partially activated are termed *stimulated* or *reparative* macrophages (M2). Partially activated macrophages contribute to (1) fibrosis and wound healing through the synthesis of mitogens such as platelet-derived growth factors, MMPs,

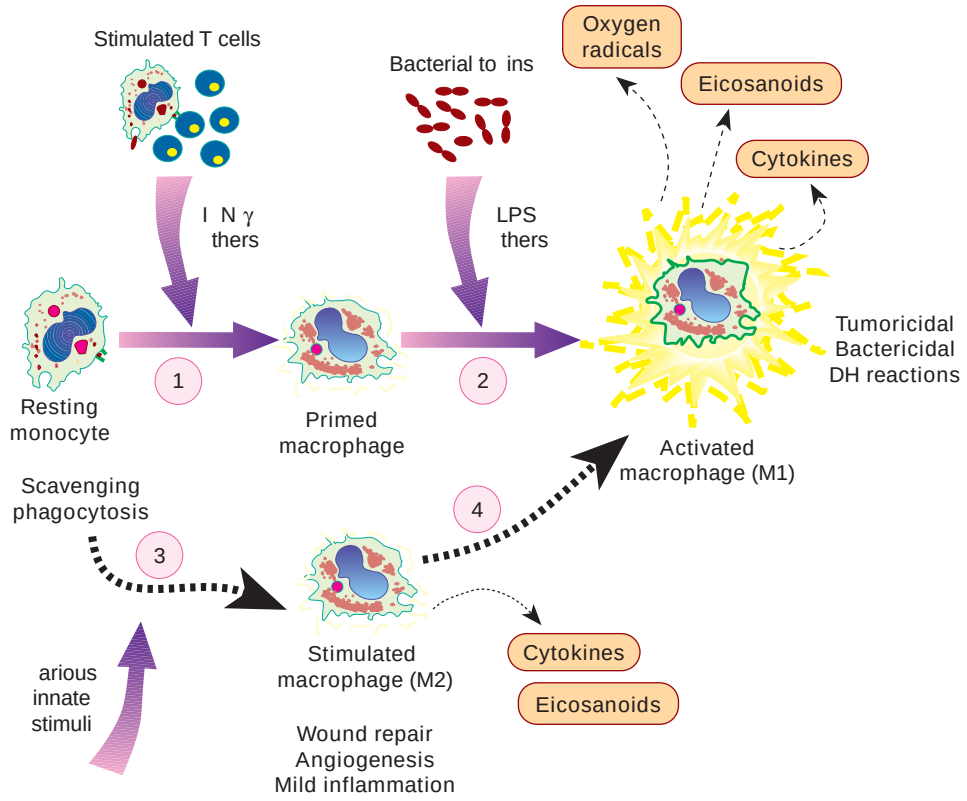

Figure 1-3 Schematic representation of macrophage activation pathway. Classically, *resting monocytes* are thought to be the principal type of noninflammatory scavenging phagocyte. **1,** Upon exposure to low levels of interferon gamma (IFN-γ) from T cells, monocytes become primed, upregulating human leukocyte antigen class II molecules for antigen presentation and performing other functions. **2,** *Fully activated macrophages (M1),* after exposure to bacterial lipopolysaccharide and interferon (classical activation), are bactericidal and tumoricidal and mediate severe inflammation. **3,** *Stimulated macrophages (M2)* result from resting monocyte activation by other innate stimuli, without exposure to IFN-γ (alternative activation). These cells are incompletely activated, producing low levels of cytokines and eicosanoids but not reactive oxygen intermediates. They participate in wound healing and angiogenesis and have immunoregulatory functions. **4,** Macrophages may switch between subsets (plasticity), depending on environmental signals. DH = delayed hypersensitivity; LPS = lipopolysaccharide. *(Illustration by Barb Cousins, modified by Joyce Zavarro.)*

and other matrix degradation factors; and to (2) angiogenesis through synthesis of angiogenic factors such as vascular endothelial growth factor.

Phagocyte Killing Mechanisms

Reactive oxygen intermediates

Under certain conditions, oxygen can undergo chemical modification into highly reactive substances with the potential to damage cellular molecules and inhibit functional properties in pathogens or host cells. Three of the most important oxygen intermediates are the

superoxide anion, hydrogen peroxide, and the hydroxyl radical. Oxygen metabolites triggered by immune responses and generated by leukocytes, especially neutrophils and macrophages, are the most important source of free radicals during inflammation. Leukocyte oxygen metabolism can be initiated by a wide variety of stimuli, including

- innate triggers, such as LPS or fMLP
- adaptive effectors, such as complement-fixing antibodies or certain cytokines produced by activated T cells
- other chemical mediator systems, such as C5a, PAF, and leukotrienes

Reactive oxygen intermediates can also be generated as part of noninflammatory cellular biochemical processes, especially by electron transport in the mitochondria, detoxification of certain chemicals, or interactions with environmental light or radiation. These reactive intermediates are highly toxic to living pathogens and damage pathogenic mediators such as exotoxins and lipids.

Reactive nitrogen products

Nitric oxide (NO) is a highly reactive chemical species. Like reactive oxygen intermediates, NO is involved in various important biochemical functions in microorganisms and host cells. At high concentrations, NO has direct cytotoxic effects on pathogens. The formation of NO depends on the enzyme nitric oxide synthetase (NOS), which is in the cytosol and dependent on NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate). Several types of NOS are known, including various forms of constitutive NOS and inducible NOS (iNOS). Activation induces enhanced production of NO in certain cells, especially macrophages, via the calcium-independent, induced synthesis of iNOS. Many innate and adaptive stimuli modulate induction of iNOS, especially cytokines and bacterial toxins.

Mediator Systems That Amplify Immune Responses

Although innate or adaptive effector responses may directly induce inflammation, in most cases this process must be amplified to produce overt clinical manifestations. Molecules generated within the host that induce and amplify inflammation are termed *inflammatory mediators,* and mediator systems include several categories of these molecules (Table 1-2). Most act on target cells through receptor-mediated processes, although some act in enzymatic cascades that interact in a complex fashion.

Table 1-2 Mediator Systems That Amplify Innate and Adaptive Immune Responses

Plasma-derived enzyme systems: complement, kinins, and fibrin
Vasoactive amines: serotonin and histamine
Lipid mediators: eicosanoids and platelet-activating factors
Cytokines
Neutrophil-derived granule products

Plasma-Derived Enzyme Systems

Complement

Complement is an important inflammatory mediator in the eye. Complement components account for approximately 5% of plasma protein and comprise more than 30 different proteins. Complement is activated by 1 of 3 pathways, and this activation generates products that contribute to the inflammatory process (Fig 1-4):

- *Classical pathway.* Activation occurs upon fixation of C1 by antigen–antibody (immune) complexes formed by IgM, IgG1, or IgG3. This pathway results in a connection between innate and adaptive immunity.
- *Alternative pathway.* Activation occurs continuously but is restricted by host complement regulatory proteins.
- *Mannose-binding lectin pathway.* This is activated by certain carbohydrate moieties on the cell wall of microorganisms.

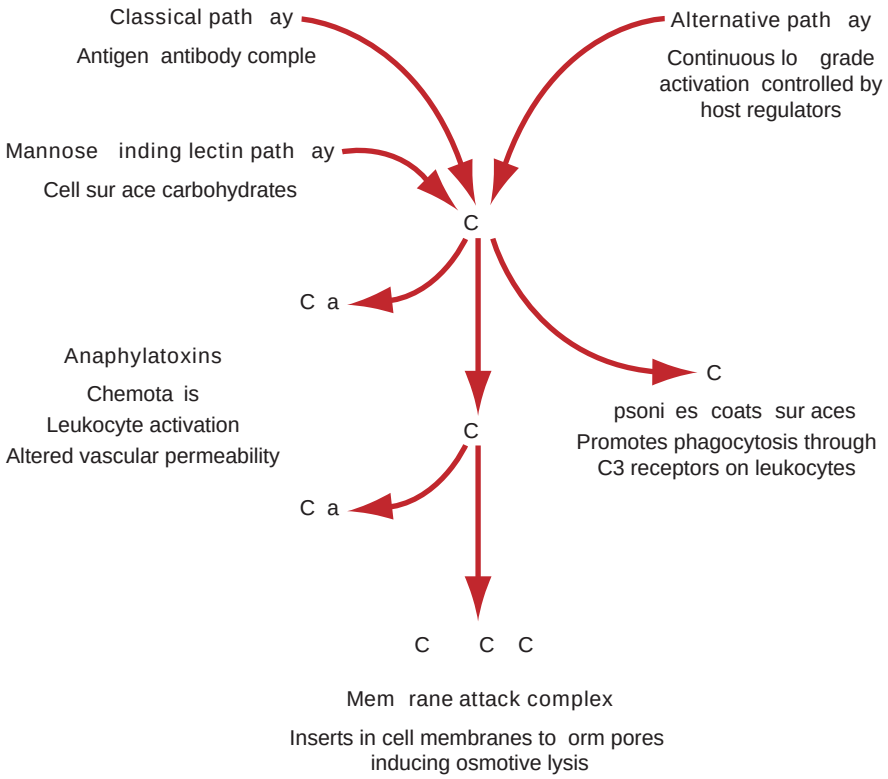

Figure 1-4 Overview of the essential intermediates of the complement pathway. C3a, C3b, C5a, and C5b are complement split products. C5b combines with intact C6, C7, C8, and C9 from the serum.

Complement serves the following 4 basic functions during inflammation:

- coats antigenic or pathogenic surfaces with C3b to enhance phagocytosis (opsonization)
- promotes lysis of cell membranes through pore formation by the membrane attack complex
- recruits neutrophils and induces inflammation through generation of the anaphylatoxins C3a and C5a
- modulates adaptive immune responses (effects on B and T cells)

Anaphylatoxin effects include chemotaxis, changes in cell adhesiveness, and degranulation and release of mediators from mast cells and platelets. C5a stimulates oxidative metabolism and the production and release of toxic oxygen radicals from leukocytes, as well as the extracellular discharge of leukocyte granule contents.

Defendi F, Thielens NM, Clavarino G, Cesbron JY, Dumestre-Pérard C. The immunopathology of complement proteins and innate immunity in autoimmune disease. *Clin Rev Allergy Immunol.* 2020;58(2):229–251.

Walport MJ. Complement. First of two parts. *N Engl J Med.* 2001;344(14):1058–1066.

Walport MJ. Complement. Second of two parts. *N Engl J Med.* 2001;344(15):1140–1144.

Fibrin and other plasma factors

Fibrin is the final deposition product of the coagulation pathway. Its deposition during inflammation promotes hemostasis, fibrosis, angiogenesis, and leukocyte adhesion. Fibrin is released from its circulating zymogen precursor, *fibrinogen,* upon cleavage by thrombin. In situ polymerization of smaller units gives rise to the characteristic fibrin plugs or clots. Fibrin dissolution is mediated by *plasmin.* Plasminogen is converted to its active form (plasmin) after cleavage by a tissue plasminogen activator. Thrombin, which is derived principally from platelet granules, is released after any vascular injury that causes platelet aggregation and release. Fibrin may be observed in severe anterior uveitis (the "plasmoid aqueous"), and it contributes to complications such as synechiae, cyclitic membranes, and traction retinal detachment.

Histamine

Histamine is present in the granules of mast cells and basophils and is actively secreted after exposure to a wide range of stimuli. Histamine acts by binding to 1 of at least 3 known types of receptors that are differentially present on target cells. The best-studied pathway for degranulation is antigen crosslinking of IgE bound to mast cell Fc IgE receptors, but many other inflammatory stimuli can induce histamine secretion, including complement, direct membrane injury, and certain drugs. Classically, histamine release has been associated with allergy. The contribution of histamine to intraocular inflammation remains subject to debate.

Lipid Mediators

Two groups of lipid molecules synthesized by stimulated cells act as powerful mediators and regulators of inflammatory responses: arachidonic acid (AA) metabolites, or

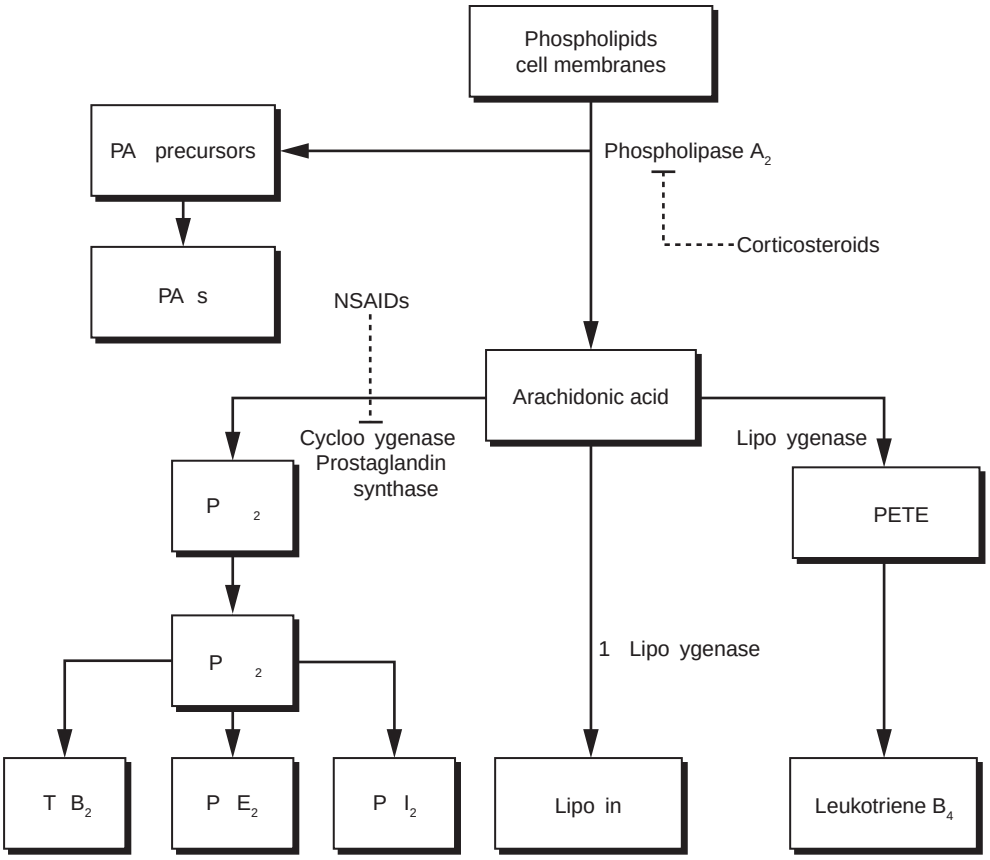

Figure 1-5 Overview of the essential intermediates of the eicosanoid and platelet-activating factor (PAF) pathways. 5-HPETE = 5-hydroperoxyeicosatetraenoic acid; NSAIDs = nonsteroidal anti-inflammatory drugs; PG = prostaglandin; TXB_2 = thromboxane B_2. *(Modified with permission from Pepose JS, Holland GN, Wilhelmus KR, eds.* Ocular Infection and Immunity. *Mosby; 1996.)*

eicosanoids, and acetylated triglycerides, usually called *platelet-activating factors (PAFs).* Both groups of molecules may be rapidly generated from the same lysophospholipid precursors by the enzymatic action of cellular phospholipases such as phospholipase A_2 (Fig 1-5).

Eicosanoids

All eicosanoids are derived from AA. AA is liberated from membrane phospholipids by phospholipase A_2, which is activated by various agonists. AA is oxidized by 2 major pathways to generate the various mediators:

- cyclooxygenase (COX) pathway, which produces prostaglandins, thromboxanes, and prostacyclins
- 5-lipoxygenase pathway, which produces 5-hydroperoxyeicosatetraenoic acid, lipoxins, and leukotrienes

Two forms of COX have been identified. COX-1 is thought to be constitutively expressed by many cells. COX-2 is inducible by various inflammatory stimuli (eg, LPS, PAF, and some cytokines). The COX-derived products are evanescent compounds induced in virtually all cells by a variety of stimuli. In general, they act in the immediate environment of their release to directly mediate many inflammatory activities. These include effects on vascular permeability, cell recruitment, platelet function, and smooth-muscle contraction. Prostaglandins may play a role in uveitic macular edema in association with anterior segment surgery or inflammation. Posterior diffusion of 1 or more of the eicosanoids through the vitreous is assumed to alter the permeability of the perifoveal capillary network, leading to macular edema. Clinical trials in humans suggest that topical treatment with COX inhibitors (eg, nonsteroidal anti-inflammatory drugs [NSAIDs]) reduces the incidence of macular edema after cataract surgery.

Derivatives of 5-lipoxygenase, an enzyme found mainly in granulocytes and some mast cells, have been detected in the brain and retina. Some leukotrienes have 1000 times the effect of histamine on vascular permeability. Another lipoxygenase product, lipoxin, is a potent stimulator of superoxide anion. Because many of the COX-derived prostaglandins downregulate the lipoxygenase pathway, NSAIDs can tilt AA metabolism toward increased production of inflammatory metabolites, leukotrienes, and lipoxins.

Platelet-activating factors

Platelet-activating factors are a family of phospholipid-derived mediators that appear to be important stimuli in the early stage of inflammation. Phospholipase A_2 metabolizes phosphocholine precursors in cell membranes, releasing AA and PAF precursors, which are acetylated into multiple species of PAF. PAF release is stimulated by various innate immune triggers, such as bacterial toxins, trauma, and cytokines. PAFs activate not only platelets but also most leukocytes, which in turn produce and release additional PAFs. PAFs function by binding to 1 or more guanosine triphosphate protein–associated receptors on target cells.

In vitro, PAFs induce an impressive repertoire of responses, including phagocytosis, exocytosis, superoxide production, chemotaxis, aggregation, proliferation, adhesion, eicosanoid generation, degranulation, and calcium mobilization, as well as diverse morphologic changes. PAFs are a major regulator of cell adhesion and vascular permeability in many forms of acute inflammation, trauma, shock, and ischemia. The precise role of PAFs in intraocular inflammation remains unknown, but synergistic interactions probably exist among PAFs, nitric oxide, eicosanoids, and cytokines. However, intravitreal injection of PAFs in animals induces an acute retinitis and photoreceptor toxicity.

Cytokines

Cytokines are soluble polypeptide mediators synthesized and released by cells for the purposes of intercellular signaling and communication, both within the innate immune system and between the innate and adaptive immune systems. Various types of intercellular signaling occur, including *paracrine* (signaling of neighboring cells at the same site), *autocrine* (stimulation of a receptor on its own surface, and *endocrine* (action on a distant site through release into the blood). Table 1-3 lists examples of cytokines associated with ocular inflammation.

Table 1-3 Cytokines of Relevance to Ocular Immunology

Family	Example	Major Cell Source	Major Target Cells	Major General Actions	Specific Ocular Actions/Clinical Relevance
Interleukins (ILs)	IL-1β	Monocytes Macrophages Neutrophils Dendritic cells T cells	Most leukocytes Various ocular cells	Induces cyclooxygenase type 2 leading to fever, vasodilatation, hypotension (shock) Promotes infiltration of inflammatory cells into extravascular space and tissues Promotes angiogenesis Induces IL-6 and IL-17 expression	Altered vascular permeability Neutrophil and macrophage infiltration Langerhans cell migration to central cornea Elevated levels in aqueous and serum in many forms of uveitis
	IL-2	Th0 or Th1 CD4 T lymphocytes	T lymphocytes B lymphocytes NK cells	Activates CD4 and CD8 T lymphocytes Induces Th1	Detectable levels in some forms of uveitis
	IL-4	Th2 CD4 T lymphocytes Basophils, mast cells	T lymphocytes B lymphocytes	Induces Th2, blocks Th1 Induces B lymphocytes to synthesize immunoglobulin E	Role in atopic and vernal conjunctivitis
	IL-5	Th2 CD4 T lymphocytes	Eosinophils	Recruits eosinophils	Role in atopic and vernal conjunctivitis
	IL-6	Monocytes Macrophages T lymphocytes Mast cells Endothelium	Most leukocytes Various ocular cells	Has many actions on B lymphocytes, including enhancement of antibody production Induces T-cell polarization Induces systemic toxicity (fever, shock, production of acute-phase proteins in liver)	Altered vascular permeability Neutrophil infiltration High levels in serum, aqueous, and vitreous in many forms of uveitis and nonuveitic diseases

Family	Example	Major Cell Source	Major Target Cells	Major General Actions	Specific Ocular Actions/Clinical Relevance
	IL-12/23 (IL-12 family)	Macrophages Dendritic cells B lymphocytes (IL-23) Endothelium (IL-23)	Naive CD4 T lymphocytes	Key Th1-inducing cytokine Activates NK cells Mediates chronic inflammation through promotion of Th17 lymphocytes	High expression in aqueous and vitreous from idiopathic uveitis cases High expression in serum in Behçet disease and VKH syndrome
	IL-17A	T lymphocytes (Th17, gamma-delta [γδ]) NK T lymphocytes	Most leukocytes Various ocular cells	Key cytokine of Th17 response, driving inflammation/tissue damage Induces proinflammatory cytokines, chemokines, and adhesion molecules Important driver of autoimmunity	High expression in patients with active uveitis, noninfectious (Behçet disease, birdshot chorioretinopathy, HLA-B27 uveitis, VKH syndrome) and infectious (toxoplasmic retinochoroiditis, viral retinitis) Leads to disruption of outer blood–retina barrier Role in infectious keratitis
α-Chemokines	IL-8/CXCL8	Many cell types	Endothelial cells Neutrophils Many others	Recruits and activates neutrophils Upregulates CAM on endothelium Chemotactic for basophils and T lymphocytes Promotes angiogenesis	High expression in inflamed eye Altered vascular permeability Neutrophil infiltration
β-Chemokines	Macrophage chemotactic protein-1/CCL2	Macrophages Endothelium RPE	Endothelial cells Macrophages T lymphocytes	Recruits and activates macrophages, some T lymphocytes	High expression in noninflamed and inflamed eyes Recruits macrophages and T lymphocytes to eye
Tumor necrosis factors (TNFs)	TNF-α or TNF-β	Macrophages T lymphocytes	Most leukocytes Various ocular cells	Tumor apoptosis Macrophage and neutrophil activation Cell adhesion and chemotaxis Fibrin deposition and vascular injury Systemic toxicity (fever, shock)	Altered vascular permeability Mononuclear cell infiltration

(Continued)

Table 1-3 *(continued)*

Family	Example	Major Cell Source	Major Target Cells	Major General Actions	Specific Ocular Actions/Clinical Relevance
Interferons (IFNs)	IFN-γ	Th1 cells NK cells	Macrophages Dendritic cells	Activates macrophages Facilitates Th1 development Mediates delayed-type hypersensitivity reactions	Neutrophil and macrophage infiltration MHC II upregulation on iris and ciliary epithelium, RPE
	IFN-α	Most leukocytes	Most parenchymal cells	Prevents viral infection of many cells Inhibits hemangioma, conjunctival intraepithelial neoplasia, and other tumors	Innate protection of ocular surface from viral infection Treatment of ocular surface neoplasms
Growth factors	Transforming growth factor β family (TGF-β)	Leukocytes RPE and NPE of ciliary body Pericytes Fibroblasts	Macrophages T lymphocytes RPE Glia Fibroblasts	Regulates immune response, suppresses T-lymphocyte and macrophage inflammatory functions Regulates wound repair: fibrosis	High expression in the noninflamed eye Regulator of immune privilege and ACAID
	Platelet-derived growth factors	Platelets Macrophages RPE	Fibroblasts Glia Many others	Fibroblast proliferation	Role in inflammatory membranes, subretinal fibrosis
	Vascular endothelial growth factor (VEGF) family	Macrophages Platelets Several retinal cells (RPE, astrocytes, Müller cells, vascular endothelium)	Vascular endothelial cells Leukocytes	Neovascularization and vascular permeability Leukocyte recruitment	Role in retinovascular disease (diabetic macular edema, exudative AMD, PDR, retinal vein occlusion, ROP) Corneal neovascularization and nerve growth
Neuropeptides	Substance P	Ocular nerves	Leukocytes	Pain	Altered vascular permeability
			Others	Altered vascular permeability	Leukocyte infiltration
	Vasoactive intestinal peptide	Ocular nerves	Leukocytes Others	Suppresses macrophage and T-lymphocyte inflammatory function	Role in ACAID and immune privilege

ACAID = anterior chamber–associated immune deviation; AMD = age-related macular degeneration; CAM = cell adhesion molecules; CCL = chemokine ligand; CXCL = C-X-C motif chemokine ligand; HLA = human leukocyte antigen; MHC = major histocompatibility complex; NK = natural killer; NPE = nonpigmented epithelium; PDR = proliferative diabetic retinopathy; RPE = retinal pigment epithelium; ROP = retinopathy of prematurity; Th = T helper; VKH = Vogt-Koyanagi-Harada.

Traditionally, investigators have divided cytokines into families with related activities, sources, and targets, using terms such as *growth factors, interleukins, lymphokines, interferons, monokines,* and *chemokines.* Thus, *growth factor* traditionally refers to cytokines that mediate cell proliferation and differentiation. The terms *interleukin* and *lymphokine* identify cytokines thought to mediate intercellular communication among lymphocytes or other leukocytes. Interferons are cytokines that limit or interfere with the ability of a virus to infect a cell. Monokines are immunoregulatory cytokines secreted by monocytes and macrophages. Chemokines are chemotactic cytokines. Although some cytokines are specific for particular cell types, most have high degrees of multiplicity and redundancy of source. For example, activated macrophages in an inflammatory site synthesize growth factors, interleukins, interferons, and chemokines.

Both innate and adaptive responses result in the production of cytokines. T lymphocytes are the classic cytokine-producing cell of adaptive immunity, but macrophages, mast cells, and neutrophils also synthesize a wide range of cytokines upon stimulation. Cytokine interactions can be additive, combinatorial, synergistic, or antagonistic. Elimination of the action of a single molecule may have an unpredictable outcome. For example, monoclonal antibodies directed against TNF-α result in substantial suppression of immune responses but also increase susceptibility to multiple sclerosis. Finally, cytokines not only act as mediators and amplifiers of inflammation in innate and adaptive immune responses but also modulate the initiation of immune responses; the function of most leukocytes is altered by preexposure to various cytokines. Thus, for many cytokines, their regulatory role may be as important as their actions as mediators of inflammation.

Targeting cytokines with monoclonal antibodies or soluble receptors has become an important and highly effective therapeutic strategy for combating inflammatory diseases (see Table 1-4 on the following pages). For example, TNF-α is a potent proinflammatory cytokine produced predominantly by activated monocytes, macrophages, and T cells that plays a role in the pathogenesis of several autoimmune diseases. Multiple TNF-α blockers are currently approved by the US Food and Drug Administration for the treatment of a number of inflammatory diseases, including noninfectious intermediate uveitis, posterior uveitis, and panuveitis (also see Chapter 6).

Neutrophils and Their Products

Neutrophils are a source of specialized products that can amplify immune responses. These products are discussed in the section "Neutrophil-derived granule products."

Table 1-4 Clinically Relevant Cytokine Inhibitors

Cytokine Targeted	Drug Examples	Drug Description	Route of Administration	FDA-Approved Indication	Ocular Relevance/Use
IL-1β	Anakinra	IL-1 receptor antagonist	Subcutaneous injection	Rheumatoid arthritis and several rare autoinflammatory conditions	Off-label use in Behçet disease
	Canakinumab	Human monoclonal Ab	Subcutaneous injection	JIA and periodic fever syndromes	Off-label use in Behçet disease
IL-6	Tocilizumab	Humanized monoclonal Ab	IV infusion or subcutaneous injection	Rheumatoid arthritis, giant cell arteritis, JIA, cytokine release syndrome	Off-label use in noninfectious intermediate, posterior, and panuveitis
IL-17A	Secukinumab	Human monoclonal Ab	Subcutaneous injection	Plaque psoriasis and psoriatic arthritis, ankylosing spondylitis	Failed to show efficacy in treatment of noninfectious intermediate, posterior, and panuveitis in randomized clinical trial
TNF-α	Adalimumab	Human monoclonal Ab	Subcutaneous injection	Rheumatoid arthritis; JIA; plaque psoriasis and psoriatic arthritis; ankylosing spondylitis; Crohn disease; ulcerative colitis; hidradenitis suppurativa; noninfectious intermediate, posterior, and panuveitis	On-label use in noninfectious intermediate, posterior, and panuveitis
	Infliximab	Chimeric monoclonal Ab	IV infusion	Crohn disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, plaque psoriasis, psoriatic arthritis	Off-label use in noninfectious intermediate, posterior, and panuveitis

Cytokine Targeted	Drug Examples	Drug Description	Route of Administration	FDA-Approved Indication	Ocular Relevance/Use
VEGF-A	Bevacizumab	Humanized monoclonal Ab	IV infusion for FDA-approved indications; intravitreal injection for off-label ocular use	Metastatic colorectal cancer and several other cancers	Off-label use in retinovascular disease (neovascular AMD, diabetic retinopathy, diabetic macular edema, ROP, RVO) and refractory uveitic macular edema
	Ranibizumab	Humanized monoclonal Ab	Intravitreal injection	Neovascular AMD, macular edema following RVO, diabetic macular edema	In addition to FDA indications, off-label use in diabetic retinopathy, myopic CNV, refractory uveitic macular edema
	Aflibercept	Fusion protein with human VEGF receptors 1 and 2 fused to human Fc	Intravitreal injection	Neovascular AMD, macular edema following RVO, diabetic macular edema, diabetic retinopathy	In addition to FDA indications, off-label use in refractory uveitic macular edema

Ab = antibody; AMD = age-related macular degeneration; CNV = choroidal neovascularization; Fc = fragment, crystallizable; FDA = Food and Drug Administration; IL = interleukin; IV = intravenous; JIA = juvenile idiopathic arthritis; ROP = retinopathy of prematurity; RVO = retinal vein occlusion; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.