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1

Basic Principles of Pathology

The most important tool that the pathologist has at his/her disposal is meaningful communication with the patient's clinician regarding the suspected diagnosis so that the pathologist can choose the appropriate strategy for processing whatever tissue or other samples are received. As will be seen in the discussion under Modern Molecular Pathology Diagnostic Techniques, there is a dizzying array of techniques at the pathologist's disposal; however, it is only through communication with the clinician that the pathologist can determine which of these techniques to utilize to best serve the patient.

INFLAMMATION

Definition

I. Inflammation is the response of a tissue or tissues to a noxious stimulus.
   A. The tissue may be predominantly cellular (e.g., retina), composed mainly of extracellular materials (e.g., cornea), or a mixture of both (e.g., uvea).
   B. The response may be localized or generalized, and the noxious stimulus may be infectious or noninfectious.

II. In a general way, inflammation is a response to a foreign stimulus that may involve specific (immunologic) or nonspecific reactions. Immune reactions arise in response to specific antigens, but they may involve other components (e.g., antibodies, T cells) or nonspecific components (e.g., natural killer [NK] cells, lymphokines).

III. There is an interplay between components of the inflammatory process and blood clotting factors that shapes the inflammatory process.

Causes

I. Noninfectious causes
   A. Exogenous causes: originate outside the eye and body, and include local ocular physical injury (e.g., perforating trauma), chemical injuries (e.g., alkali), or allergic reactions to external antigens (e.g., conjunctivitis secondary to pollen).
   B. Endogenous causes: sources originating in the eye and body, such as inflammation secondary to cellular immunity (phacoanaphylactic endophthalmitis [phacoantigenic uveitis]); spread from continuous structures (e.g., the sinuses); hematogenous spread (e.g., foreign particles); and conditions of unknown cause (e.g., sarcoidosis).

II. Infectious causes include viral, rickettsial, bacterial, fungal, and parasitic agents.

Phases of Inflammation

(Table 1.1 lists the actions of the principal mediators of inflammation.)

I. Acute (immediate or shock) phase (Fig. 1.1)
   A. Five cardinal signs: (1) redness (rubor) and (2) heat (calor)—both caused by increased rate and volume of blood flow; (3) mass (tumor)—caused by exudation of fluid (edema) and cells; (4) pain (dolor) and (5) loss of function (functio laesa)—both caused by outpouring of fluid and irritating chemicals. Table 1.2 lists the roles of various mediators in the different inflammatory reactions.
   B. The acute phase is related to histamine release from mast cells and factors released from plasma (kinin, complement, and clotting systems).
      1. *Histamine* is found in the granules of mast cells, where it is bound to a heparin–protein complex. Serotonin (5-hydroxytryptamine), found in platelets and some neuroendocrine cells, has a similar effect to histamine.
      2. The *kinins* are peptides formed by the enzymatic action of kallikrein on the α_2-globulin kininogen. Kallikrein is activated by factor XIIa, which is the active form of the coagulation factor XII (*Hageman factor*). Factor XIIa converts plasma prekallikrein into kallikrein. Plasmin also can activate Hageman factor.
      3. *Plasmin*, the proteolytic enzyme responsible for fibrinolysis, has the capacity to liberate kinins from their precursors and to activate kallikrein, which brings about the formation of plasmin from plasminogen. Plasmin cleaves C3 complement protein, resulting in the formation of C3 fragments. It also breaks down fibrin to form fibrin split products.
      4. The *complement system* (see Table 1.3, which lists the complement molecules found in the normal eye, and Table 1.4, which lists the complement molecules found in diseased eyes) consists of almost 60 proteins present in blood plasma, on the cell surfaces, or within the cell. Its vital nature is evidenced by the fact that it has been preserved by evolution for more than a billion years.

TABLE 1.1 The Actions of the Principal Mediators of Inflammation

Mediator	Principal Sources	Actions
Cell-Derived		
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Serotonin	Platelets	Vasodilation, increased vascular permeability
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion and activation
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Reactive oxygen species	Leukocytes	Killing of microbes, tissue damage
Nitric oxide	Endothelium, macrophages	Vascular smooth muscle relaxation, killing of microbes
Cytokines (TNF, IL-1)	Macrophages, endothelial cells, mast cells	Local endothelial activation (expression of adhesion molecules), fever/pain/anorexia/hypotension, decreased vascular resistance (shock)
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation
Plasma Protein-Derived		
Complement products (C5a, C3a, C4a)	Plasma (produced in liver)	Leukocyte chemotaxis and activation, vasodilation (mast cell stimulation)
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain
Proteases activated during coagulation	Plasma (produced in liver)	Endothelial activation, leukocyte recruitment

IL-1, interleukin-1; MAC, membrane attack complex; TNF, tumor necrosis factor.
(Reproduced from Table 2.4, Kumar R, Abbas A, DeLancey A et al.: *Robbins and Cotran Pathologic Basis of Disease,* 8th edn. Philadelphia, Saunders. © 2010 by Saunders, an imprint of Elsevier Inc.)

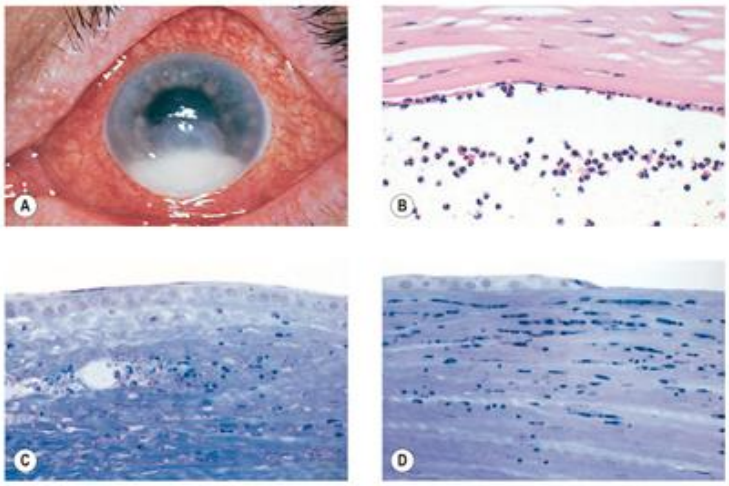

Fig. 1.1 Acute inflammation. **A,** Corneal ulcer with hypopyon (purulent exudate). Conjunctiva hyperemic. **B,** Polymorphonuclear leukocytes (PMNs) adhere to corneal endothelium and are present in the anterior chamber as a hypopyon (purulent exudate). **C,** Leukocytes adhere to limbal, dilated, blood-vessel wall (margination) and have emigrated through endothelial cell junctions into edematous surrounding tissue. **D,** PMNs in corneal stroma do not show characteristic morphology but are recognized by "bits and pieces" of nuclei lining up in a row. (**C** and **D** are thin sections from rabbit corneas six hours post-corneal abrasion.)

TABLE 1.2 Role of Mediators in Different Reactions of Inflammation

Role in Inflammation	Mediators
Vasodilation	Prostaglandins
	Nitric oxide
	Histamine
Increased vascular permeability	Histamine and serotonin
	C3a and C5a (by liberating vasoactive amines from mast cells, other cells)
	Bradykinin
	Leukotrienes C_4, D_4, E_4
	PAF
	Substance P
Chemotaxis, leukocyte recruitment and activation	TNF, IL-1
	Chemokines
	C3a, C5a
	Leukotriene B_4
	(Bacterial products; e.g., *N*-formyl methyl peptides)
Fever	IL-1, TNF
	Prostaglandins
Pain	Prostaglandins
	Bradykinin
Tissue damage	Lysosomal enzymes of leukocytes
	Reactive oxygen species
	Nitric oxide

IL-1, interleukin-1; PAF, platelet-activating factor; TNF, tumor necrosis factor.
(Reproduced from Table 2.7, Kumar R, Abbas A, DeLancey A et al.: *Robbins and Cotran Pathologic Basis of Disease*, 8th edn. Philadelphia, Saunders. © 2010 by Saunders, an imprint of Elsevier Inc.)

TABLE 1.3 Complement Molecules Found in the Normal Eye

Complement Molecules Expressed in the Healthy Eye	Eye-Associated Remarks
Complement System Activators	
Amyloid precursor proteins (APP)	Retina
C-reactive protein (CRP)	Retina
Complement Proteins	
C1q, C2, C3	Cornea, choroid, inner retina, sclera, optic nerve, retinal pigmented epithelium (RPE) cell
C4	Sclera
C5–8	Cornea, scleral tissue
C9	Soft drusen from non-AMD eyes, retina, optic nerve
C5b–9	Bruch's membrane, increase with age in non-AMD eyes
Factor B	Cornea, sclera
Complement Regulators	
Factor H	Cornea, sclera, iris, ciliary body, retina, choroidal tissue outside Bruch's membrane, optic nerve
Factor H-like protein 1 (FHL-1)	Bruch's membrane
C1 inhibitor (C1-INH)	Cornea
CD46 (MCP)	Cornea and corneal limbus, vitreous humor, RPE basolateral surface, photoreceptors
CD55 (DAF)	Cornea and corneal limbus, conjunctiva, iris, ciliary body, vitreous humor, retinal nerve fiber layer (NFL) and photoreceptors
CD59 (protectin)	Cornea and corneal limbus, conjunctiva, iris, ciliary body, choroid, vitreous humor, vessels in the inner retina
Vitronectin	Soft drusen from non-AMD eyes
Clusterin	Soft drusen from non-AMD eyes
Complement Receptors	
Complement receptor-1 (CR1)	RPE apical surface
C3aR	Retinal ganglion cells, NFL
C5aR	Inner plexiform layer (IPL), Müller cells, NFL

AMD, age-related macular degeneration; RPE, retinal pigment epithelium.
(From Mohlin et al.: The link between morphology and complement in ocular disease. *Mol Immunol* 89:84–99, 2017. Table 1. Elsevier.)

a. Initially named because it was seen to "complement" antibody and cell-mediated immune defenses against microbes.
b. Classic functions: Fig. 1.2 highlights some of the myriad functions performed by complement.
   1) Removal of immune (antigen–antibody) complexes.
   2) Labeling (opsonization) of foreign antigens for enhanced removal by phagocytes.
   3) Recruitment and activation of nearby leukocytes.
   4) Direct cytolysis of invading microorganisms.
c. Performs multiple functions in addition to those "classically" ascribed to it.
d. Complement achieves its effect through a cascade of the separate components working in coordination and in specific sequences leading through activation of C3. (Fig. 1.3 is a schematic representation of the three primary routes or pathways of complement cascade activation through C3.)
   1) The three pathways leading to activation of C3 are:
      a) Classical pathway.
      b) Lectin pathway.
      c) Alternative pathway.
   2) Cleavage of C3 produces the active fragments C3a and C3b.
      a) C3a is anaphylatoxin leading to chemotactic and proinflammatory responses.
      b) C5a also is an anaphylatoxin.
      c) C3b results in opsonization of foreign surfaces.
   3) Thus, C3 has a major role in complement activation and generation of immune responses.

TABLE 1.4 Complement Molecules Found in the Human Diseased Eye, i.e., in Age-Related Macular Degeneration (AMD), Glaucoma, Neuromyolitis Optica (NMO) and in Uveitis

Complement Molecules Expressed in the Diseased Eye	Eye Disease–Associated Remarks
Complement System Activators	**Age-Related Macular Degeneration (AMD)**
Amyloid precursor proteins (APP)	Drusen
C-reactive protein (CRP)	Drusen, choroid
Immunoglobulin	Drusen
Lipoprotein	Drusen
Complement Proteins/Activation Products	
C1q	Drusen
Mannose binding protein (MBL)	Drusen
C2[a]	
C3[a], C3c, C3d, C3dg, C3b, iC3b, Bb	Choroid, drusen, retinal pigmented epithelial (RPE) cell
C5b–9 (MAC) and sC5b–9[a]	Drusen, RPE, choroid, macula
Factor B[a]	Drusen, choroid
Factor D[a]	Drusen, retina
Complement Regulators	
Factor I[a]	Drusen, inner retina
Factor H[a]	Drusen, retinal pigmented epithelial (RPE) cell, choroid, macula
FHL-1	Drusen, choroid
Complement receptor 1 (CR1, CD35)	Drusen, RPE
CD46 (MCP)	Drusen, choroidal vessels, basolateral RPE
Vitronectin	Drusen, RPE
Clusterin	Drusen
Complement Anaphylatoxins	
C3a	Aqueous humor, drusen
C5a	Drusen
	Glaucoma
Complement Molecules Expressed in the Diseased Eye	**Eye Disease–Associated Remarks**
Complement System Activators	
Immunoglobulin	Retina, optic nerve
Complement Proteins/Activation Products	
C1q	Retina, ganglion cells (GCL) and nerve fiber layer (NFL)
C3, C3b	Retina, GCL and NFL
C5b-9 (MAC)	Retina, GCL
Complement Regulators	
Factor H	GCL
	Uveitis
Complement System Activators	
Immunoglobulin	Ocular proteins
Complement Proteins/Activation Products	
C3c, C3d	Aqueous humor
C4a	Aqueous humor
Factor B and Bb	Aqueous humor
Complement Anaphylatoxins	
C3a, C5a	Aqueous humor
	Neuromyelitis optica (NMO)
Complement System Activators	
Immunoglobulin	Optic nerve

[a]Complement-associated genes connected with AMD: (Adamus et al., 2017; Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Heckner et al., 2010; Klein et al., 2005; Gold et al., 2006; Maller et al., 2007; Park et al., 2009) and uveitis: (Thompson et al., 2013; Yang et al., 2011, 2013; Xu et al., 2015).
(From Mohlin et al., The link between morphology and complement in ocular disease. *Mol Immunol* 89:84–99, 2017. Table 2. Elsevier.)

e. C1 has been called the "defining component" of the classical complement pathway.
   1) Functions as a molecular scaffold for binding of other complement components.
   2) Activates and cleaves complement components to continue the complement cascade.
   3) Helps to trigger Wnt receptor signaling.
   4) Participates in the process of apoptosis.
   5) Cleaves MHC class I molecule and other proteins.
   6) Can adapt to multiple molecular and cellular processes besides the complement system.

f. Complement plays major roles in immune defense against microorganisms and in clearing damaged host components.
   1) It responds to recognition of pathogen-associated molecular patterns (PAMPs) when they bind to host pattern-recognition receptors (PRRs) and/or internally produced danger-associated molecular patterns (DAMPs).

g. Activation of complement pathways results in a proinflammatory response that includes the generation of membrane attack complexes (MACs), which mediate cell lysis, the release of chemokines to attract inflammatory cells to the site of damage, and the enhancement of capillary permeability. (See Fig. 1.3 for the steps leading to activation of MAC.)
   1) Composed of five terminal complement proteins: C5b, C6, C7, C8, and C9. Multiple C9 molecules may be involved.
   2) There are numerous levels regulating the activity of MAC and protecting heathy cells from attack. In fact, control of the system is the responsibility of almost half of its components.

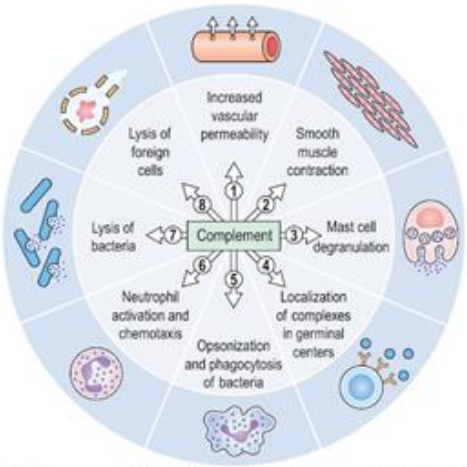

Fig. 1.2 Summary of the actions of complement and its role in the acute inflammatory reaction. Note how the elements of the reaction are induced. Increased vascular permeability (1) due to the action of C3a and C5a on smooth muscle (2) and mast cells (3) allows exudation of plasma protein. C3 facilitates both the localization of complexes in germinal centers (4) and the opsonization and phagocytosis of bacteria (5). Neutrophils, which are attracted to the area of inflammation by chemotaxis (6), phagocytose the opsonized microorganisms. The membrane attack complex, C5–C9, is responsible for the lysis of bacteria (7) and other cells recognized as foreign (8). (Adapted with permission from Roitt IM, Brostoff J, Male DK: *Immunology*, 2nd edn. London, Gower Medical. © Elsevier 1989.)

   a) Disorders resulting from impaired regulation of complement are termed complementopathies.

h. Complement proteins opsonize or lyse cells. Therefore, they may injure healthy tissue, particularly when there is a defect in complement regulation.
i. Complement is important in such diseases as macular degeneration, rheumatoid arthritis, multiple sclerosis, Alzheimer's disease, schizophrenia, and angioedema.
j. T cells and other cell types contain multiple complement components, which have been called the "complosome" in analogy to the inflammasome, which will be discussed later in this chapter. (Fig. 1.4 provides an overview of the multiple ways in which the cell complosome and other complement components may impact key cell processes when faced with various challenges.)
k. Other immune system cells that may produce or be involved in complement function are polymorphonuclear leukocytes, mast cells, monocytes, macrophages, dendritic cells, natural killer (NK) cells, and B cells.
l. Plays a role in adaptive immune response involving T and B cells, and functions as a bridge between innate and adaptive immunity.
m. Helps maintain tissue homeostasis and cellular integrity, and functions in tissue regeneration. Also functions in early sperm–egg interactions in fertilization, regulation of epiboly and organogenesis, and in refinement of cerebral synapses.
n. The complement system is implicated in multiple ocular diseases including age-related macular degeneration, glaucoma, and neuromyelitis optica (Table 1.4 lists elements of the complement system and how they may be involved in these disorders).
o. Complement system, components and their genetic deficiency.
   1) Deficiency of early components of the classical pathway (C1q, C1r/s, C2, C4, and C3) is associated with autoimmune diseases resulting from failure of clearance of immune complexes and apoptotic materials and impairment of humoral response.
   2) Deficiencies of mannan-binding lectin and the early components of the alternative (factor D and properdin) and terminal pathways (from C3 onward components C5, C6, C7, C8, and C9) increase susceptibility to infections and to their recurrence.
   3) See also the discussion of monogenic autoinflammatory syndromes later in this chapter.
p. Activation of complement in the tumor microenvironment enhances tumor growth and increases metastasis.

5. *Prostaglandins* (*prostanoids*), which have both inflammatory and anti-inflammatory effects, are 20-carbon, cyclical, unsaturated fatty acids with a 5-carbon ring and two aliphatic side chains.
   a. They are produced by mast cells, macrophages, endothelial cells, and others.
   b. With leukotrienes, they are designated eicosanoids. Leukotrienes are metabolized through the lipoxygenase pathway and prostaglandins through the cyclooxygenase pathway.
   c. Active in vascular and systemic reactions of inflammation, oxidative stress, and physiologic functions.
   d. Cyclooxygenase helps catalyze the biosynthesis of prostaglandins from arachidonic acid.
   e. Prostaglandins, cytokines, and leukotrienes function to dilate lymphatics at a site of injury.
   f. Prostaglandins play an important role in nociception and pain.
6. Major histocompatibility complex (MHC), called the human leukocyte antigen (HLA) complex in humans, is critical to the immune response.
   a. HLAs are present on all nucleated cells of the body and platelets.

> The HLA region is on autosomal chromosome 6. In practice, the blood lymphocytes are the cells tested for HLA.

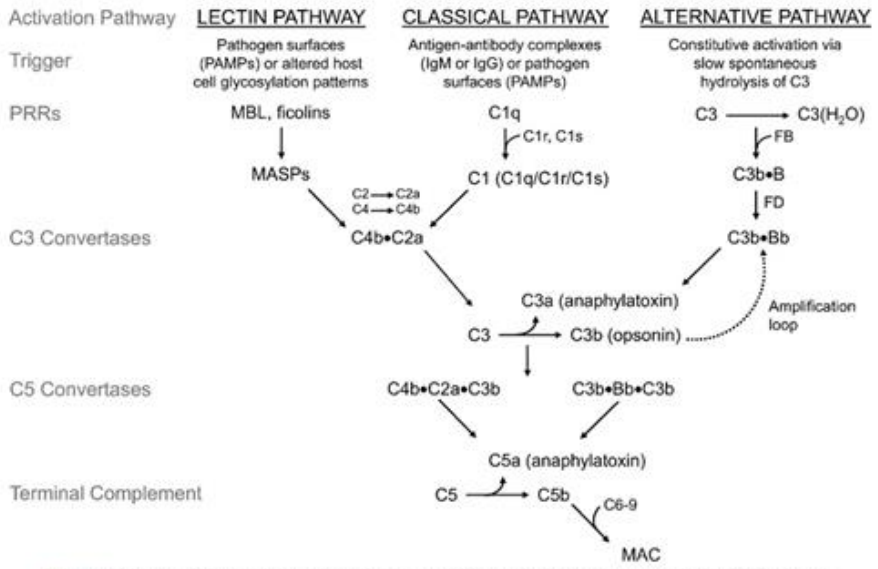

Fig. 1.3 Schematic of the complement cascade. The three primary routes for activation of complement are: (1) the lectin pathway (LP), (2) the classical pathway (CP), and (3) the alternative pathway (AP). The LP and CP are activated when specific triggers are recognized by host pattern-recognition receptors (PRRs). The AP is constitutively active. Initial activation through the LP or CP generates a shared C3 convertase (C4b•C2a). In the AP, C3b pairs with factor B (FB) to form the AP proconvertase (C3b•B), which is processed by factor D (FD) to form the AP C3 convertase (C3b•Bb). Both types of C3 convertases cleave C3 to generate C3a and C3b. C3a is an anaphylatoxin, a substance that promotes an inflammatory response. C3b that lands on the surface of a healthy host cell is quickly inactivated; C3b that attaches to the surface of a pathogen or altered host cell triggers a rapid amplification loop to generate more C3b, resulting in opsonization. C3b also complexes with the C3 convertases to form the C5 convertases (C4b•C2a•C3b and C3b•Bb•C3b). In the terminal complement cascade, C5 convertases cleave C5 into C5a (an anaphylatoxin) and C5b. C5b combines with C6–9 to form the membrane attack complex (MAC), also referred to as the terminal complement complex (TCC). Regulatory factors act at various stages of the cascade to control complement activation via their decay accelerating activity and/or cofactor activity. Additional abbreviations: MASPs, mannose-binding lectin-associated serine proteases; MBL, mannose-binding lectin; PAMPs, pathogen-associated molecular patterns. (From Baines AC, Brodsky RA: Complementopathies. *Blood Rev* 31:213–223, 2017. Figure 1. Elsevier.)

b. The three genetic loci belonging to HLA class I are designated by the letters HLA-A, HLA-B, and HLA-C. Class II MHC molecules are encoded at the locus HLA-D with three subregions HLA-DP, HLA-DQ, and HLA-DR.
   1) Class I MHC molecules display proteins derived from foreign antigens, which are recognized by $CD8^+$ T lymphocytes.
   2) Class II MHC molecules present antigens that are contained in intracellular vesicles and derived from foreign organisms and soluble proteins.

c. A tentatively identified specificity carries the additional letter "W" (workshop) and is inserted between the locus letter and the allele number—for example, HLA-BW 15.

d. The HLA system is the main human leukocyte isoantigen system and the major human histocompatibility system.
   1) HLA-B 27 is positive in a high percentage of young women who have acute anterior uveitis and in young men who have ankylosing spondylitis or Reiter's disease.
   2) HLA-B 51 is strongly associated with Behçet's disease.

7. *Nonspecific soluble mediators* of the immune system include cytokines, such as interleukins, which are mediators that act between leukocytes, interferons (IFNs), colony-stimulating factors (CSFs), tumor necrosis factor (TNF), transforming growth factor-β, and lymphokines (produced by lymphocytes).