# 2

Inflammation

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Inflammation is the reaction of a tissue and its microcirculation to a pathogenic insult. It is characterized by elaboration of inflammatory mediators and movement of fluid and leukocytes from the blood into extravascular tissues. This response localizes and eliminates altered cells, foreign particles, microorganisms, and antigens and paves the way for the return to normal structure and function.

The clinical signs of inflammation, termed *phlogosis* by the Greeks and *inflammation* in Latin, were described in classical times. In the second century AD, the Roman encyclopedist Aulus Celsus described the four cardinal signs of inflammation, namely, **rubor** (redness), **calor** (heat), **tumor** (swelling), and **dolor** (pain). These features correspond to inflammatory events of vasodilation, edema, and tissue damage. According to medieval concepts, inflammation represented an imbalance of various "humors," including blood, mucus, and bile. Modern appreciation of the vascular basis of inflammation began in the 18th century with John Hunter, who noted dilation of blood vessels and appreciated that pus was accumulated material derived from the

blood. Rudolf Virchow first described inflammation as a reaction to prior tissue injury. To the four cardinal signs he added a fifth: **functio laesa** (loss of function). Virchow's pupil Julius Cohnheim was the first to associate inflammation with emigration of leukocytes through the walls of the microvasculature. At the end of the 19th century, the role of phagocytosis in inflammation was emphasized by the eminent Russian zoologist Eli Metchnikoff. Finally, the importance of chemical mediators was described in 1927 by Thomas Lewis, who showed that histamine and other substances increased vascular permeability and caused migration of leukocytes into extravascular spaces. More recent studies have elucidated the molecular and genetic bases of acute and chronic inflammation.

Overview of inflammation

The primary function of the inflammatory response is to eliminate a pathogenic insult and remove injured tissue components, thereby allowing tissue repair to take place. The body attempts to contain
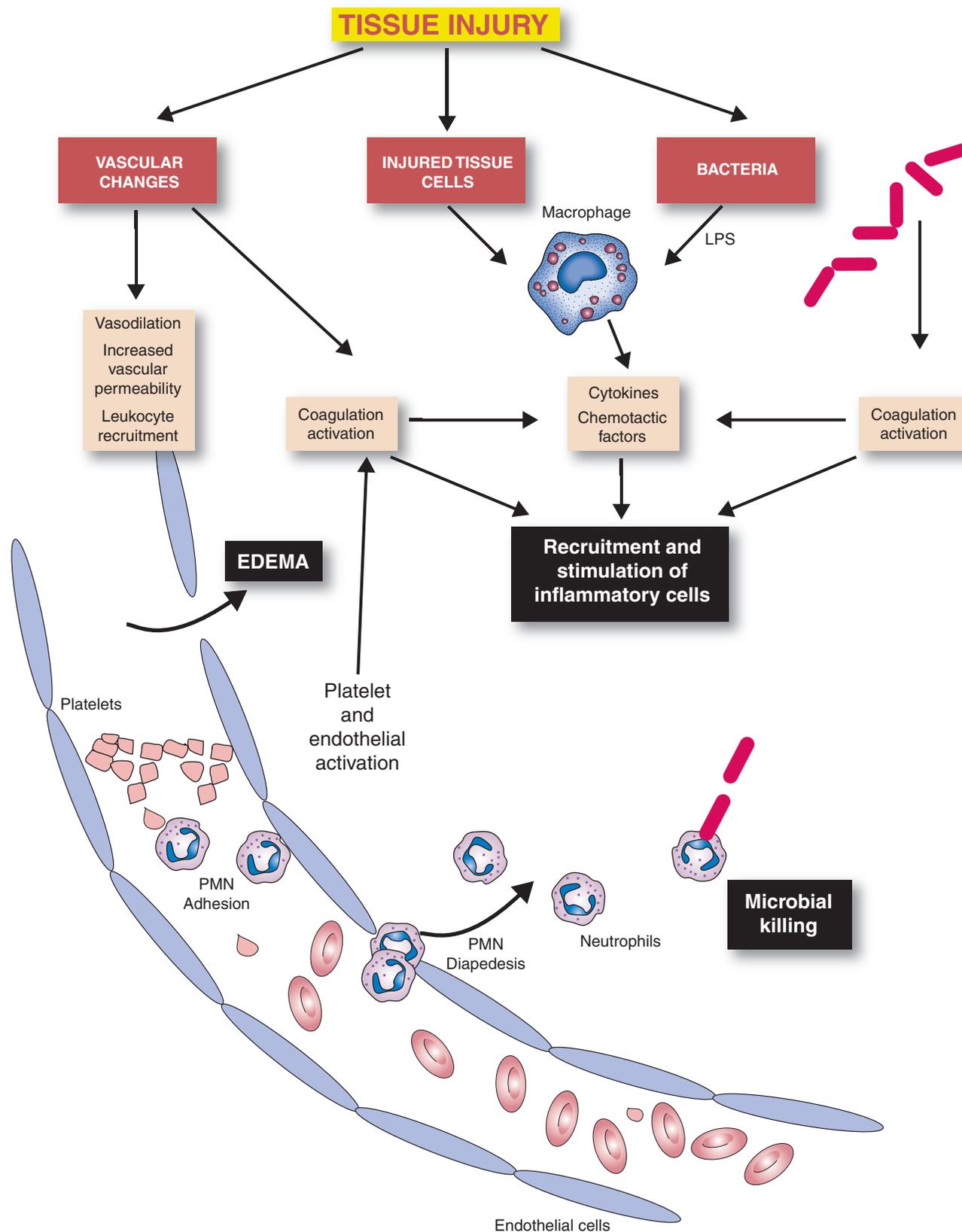
FIGURE 2-1. **The inflammatory response to injury.** Chemical mediators and cells are released from plasma following tissue injury. Vasodilation and vascular injury lead to leakage of fluid into tissues (edema). Platelets are activated to initiate clot formation and hemostasis, and to increase vascular permeability via histamine release. Vascular endothelial cells contribute to clot formation, retract to allow increased vascular permeability, and anchor circulating neutrophils via their adhesion molecules. Microbes initiate activation of the complement cascade, which, along with soluble mediators from macrophages, recruit neutrophils to the site of tissue injury. Neutrophils eliminate microbes and remove damaged tissue so that repair can begin. PMN = polymorphonuclear neutrophil.

or eliminate offending agents, thereby protecting tissues, organs, and ultimately the whole body from damage. Specific cells are imported to attack and destroy injurious agents (e.g., infectious organisms, toxins, or foreign material), enzymatically digest and remove them, or wall them off. During this process, damaged cells and tissues are digested and removed to allow repair to take place. The response to many damaging agents is immediate and stereotypic. The character of the inflammatory response is "modulated" depending upon several factors, including the nature of the offending agent, duration of the insult, extent of tissue damage, and microenvironment.

- **Initiation** of the inflammatory response results in activation of soluble mediators and recruitment of inflammatory cells to the area. Molecules are released from the offending agent, damaged cells, and the extracellular matrix, which alters the permeability of adjacent blood vessels plasma, soluble molecules, and circulating inflammatory cells. This stereotypic, immediate response leads to rapid flooding of the injured tissue with fluid, coagulation factors, cytokines, chemokines, platelets, and inflammatory cells, neutrophils in particular (Figs.2-1 and 2-2). This overall process is termed **acute inflammation.**

- **Amplification** depends upon the extent of injury and activation of mediators such as kinins and complement components. Additional leukocytes and macrophages are recruited to the area.

- **Destruction** of the damaging agent brings the process under control. Enzymatic digestion and phagocytosis reduce or eliminate foreign material or infectious organisms. At the same time, damaged tissue components are also removed, and debris is cleared away, paving the way for repair to begin (see Chapter 3).

- **Termination** of the inflammatory response is mediated by intrinsic anti-inflammatory mechanisms that limit tissue damage
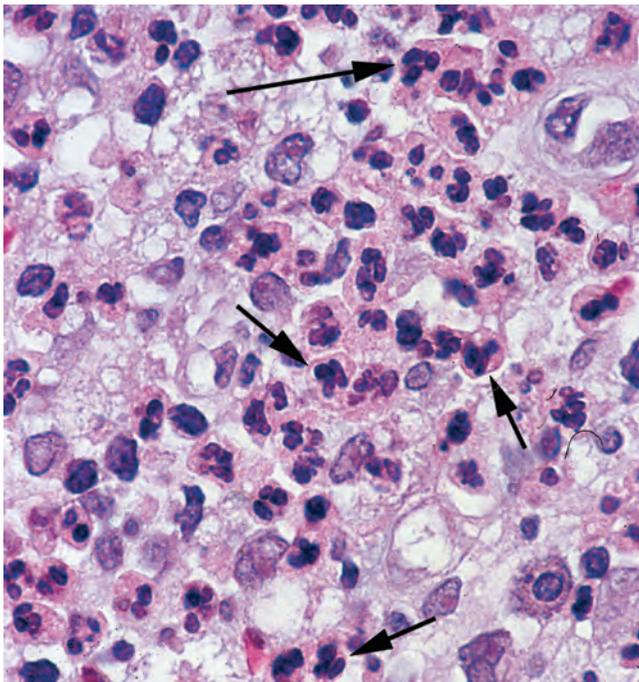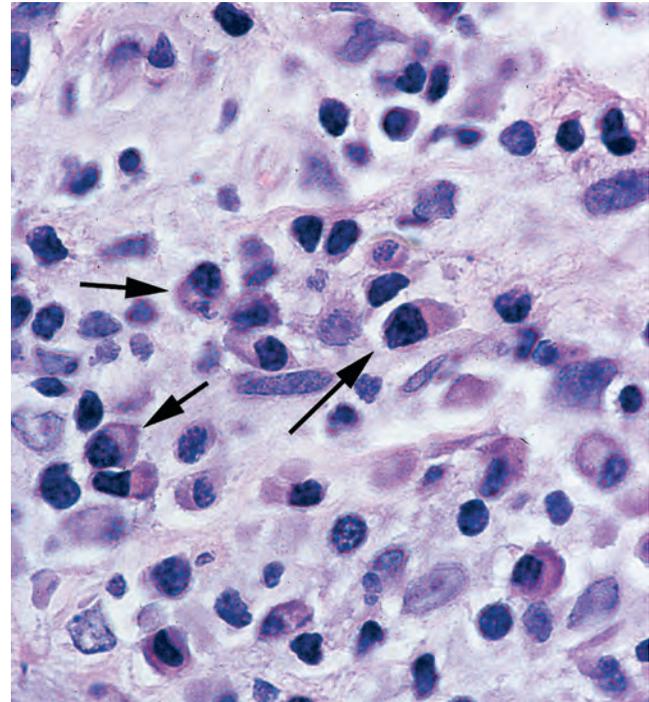
FIGURE 2-3. **Chronic inflammation.** Lymphocytes, plasma cells *(arrows),* and a few macrophages are present.

and allow for repair and a return to normal physiological function. Alternatively, depending upon the nature of the injury and the specific inflammatory and repair response, a scar may develop in place of normal tissue. Importantly, intrinsic mechanisms are in place to terminate the inflammatory process; to prevent further influx of fluid, mediators, and inflammatory cells; and to avoid digesting normal cells and tissue.

Certain types of injury trigger a sustained immune and inflammatory response with the inability to clear injured tissue and foreign agents. Such a persistent response is termed **chronic inflammation.** Chronic inflammatory infiltrates are composed largely of lymphocytes, plasma cells, and macrophages (Fig. 2-3). Acute and chronic inflammatory infiltrates often coexist.

Although inflammation usually works to defend the body, inflammation may also be harmful. Acute inflammatory responses may be exaggerated or sustained, with or without clearance of the offending agent. Tissue damage may result: witness the ravages of bacterial pneumonia owing to acute inflammation or joint destruction in septic arthritis. Chronic inflammation may also damage tissue and lead to scarring and loss of function. Indeed, chronic inflammation is the basis for many degenerative diseases.

Impaired inflammatory responses may lead to uncontrolled infection, as in immunocompromised hosts. Several congenital diseases are characterized by deficient inflammatory responses due to defects in inflammatory cell function or immunity.

Acute Inflammation

The acute inflammatory response begins with direct injury or stimulation of cellular or structural components of a tissue, including:

- Parenchymal cells
- Microvasculature

FIGURE 2-2. **Acute inflammation with densely packed polymorphonuclear neutrophils (PMNs) with multilobed nuclei *(arrows).***

- Tissue macrophages and mast cells
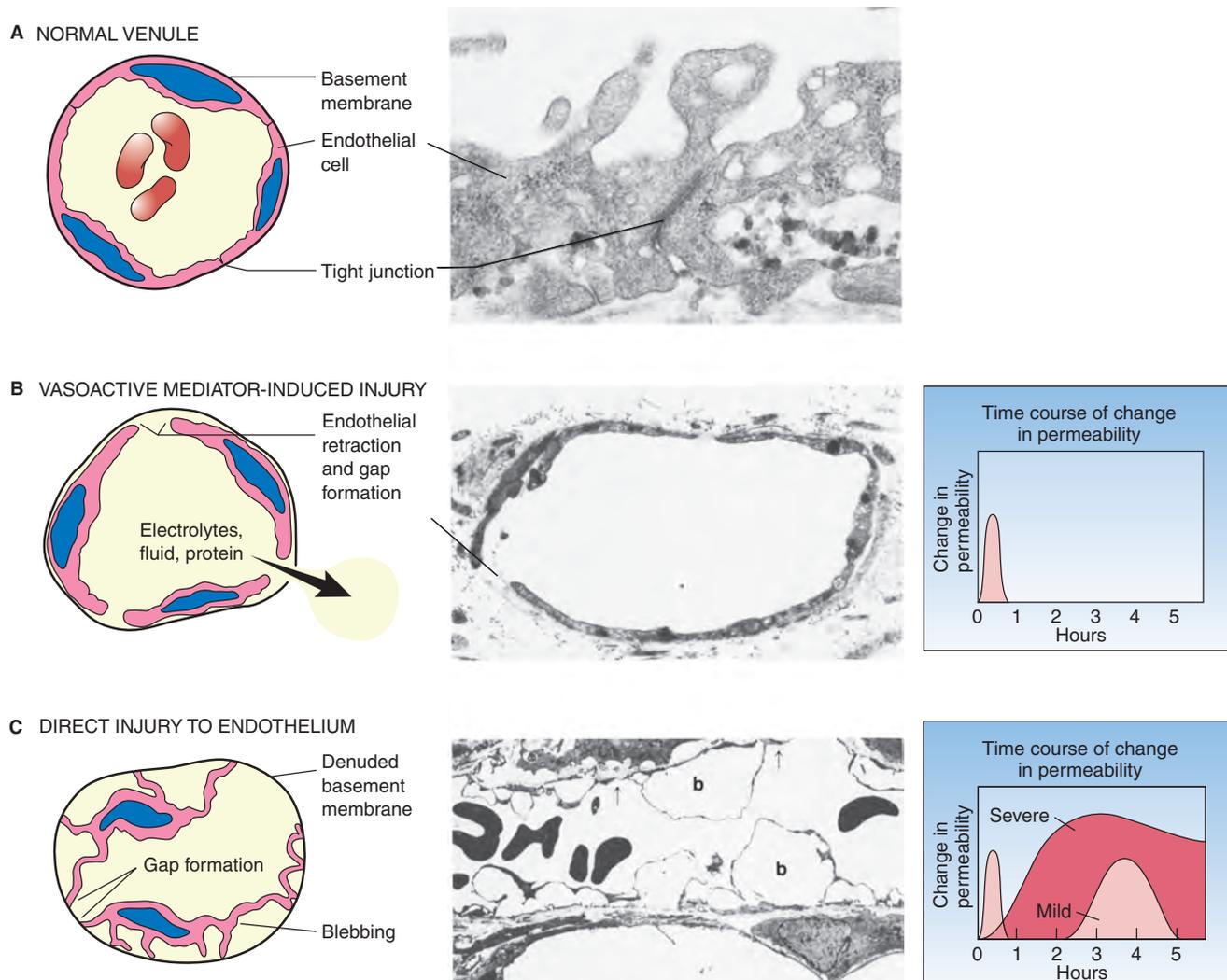- Mesenchymal cells (e.g., fibroblasts)
- Extracellular matrix (ECM)

Vascular Events After Initiation

A sequence of events occurs following initiation of acute inflammation:

- **Increased vascular permeability leads to accumulation of fluid and plasma components in tissues affected by inflammation.** Fluid exchange occurs normally between intravascular and extravascular spaces, with the endothelium forming a permeability barrier. Endothelial cells are connected to each other by tight junctions and separated from the tissue by a limiting basement membrane (Fig. 2-4). *Disruption of this barrier function is a hallmark of acute inflammation.* One of the earliest responses to tissue injury occurs at the level of capillaries and postcapillary venules. Specific inflammatory mediators are produced at the site of injury and act directly upon blood vessels to increase vascular permeability. Vascular leakage is caused by endothelial cell

contraction, endothelial cell retraction, and alterations in transcytosis. Endothelial cells are also damaged, either directly by endothelial injury or indirectly by leukocyte-mediated damage. The loss of the permeability barrier may be extensive and leakage of fluid and cells into the extravascular space, termed **edema** (see Fig. 2-4).

- **Intravascular stimulation of platelets and inflammatory cells, and release of soluble mediators.** Specific inflammatory mediators produced at sites of injury activate platelets and intravascular inflammatory cells. Kinins, complement, and components of the coagulation cascade are activated (see Fig. 2-1 and Fig 2-5), further increasing vascular permeability and edema.

- **Recruitment of neutrophils to the injured site.** Chemotactic factors then recruit leukocytes, especially neutrophils, from the vascular compartment into the injured tissue (see Figs. 2-1 and 2-2). Once present in tissues, recruited leukocytes initiate the process of eliminating the offending agents so damaged components can be removed and tissue repair can commence. These cells secrete additional mediators, which either enhance or inhibit the inflammatory response.

A NORMAL VENULE
- Basement membrane
- Endothelial cell
- Tight junction

B VASOACTIVE MEDIATOR-INDUCED INJURY
- Endothelial retraction and gap formation
- Electrolytes, fluid, protein
- Time course of change in permeability

C DIRECT INJURY TO ENDOTHELIUM
- Denuded basement membrane
- Gap formation
- Blebbing
- Time course of change in permeability
  - Severe
  - Mild

FIGURE 2-4. **Responses of the microvasculature to injury. A.** The wall of the normal venule is sealed by tight junctions between adjacent endothelial cells. **B.** During mild vasoactive mediator-induced injury, the endothelial cells separate and permit the passage of the fluid constituents of the blood. **C.** With severe direct injury, the endothelial cells form blebs *(b)* and separate from the underlying basement membrane. Areas of denuded basement membrane *(arrows)* allow a prolonged escape of fluid elements from the microvasculature.
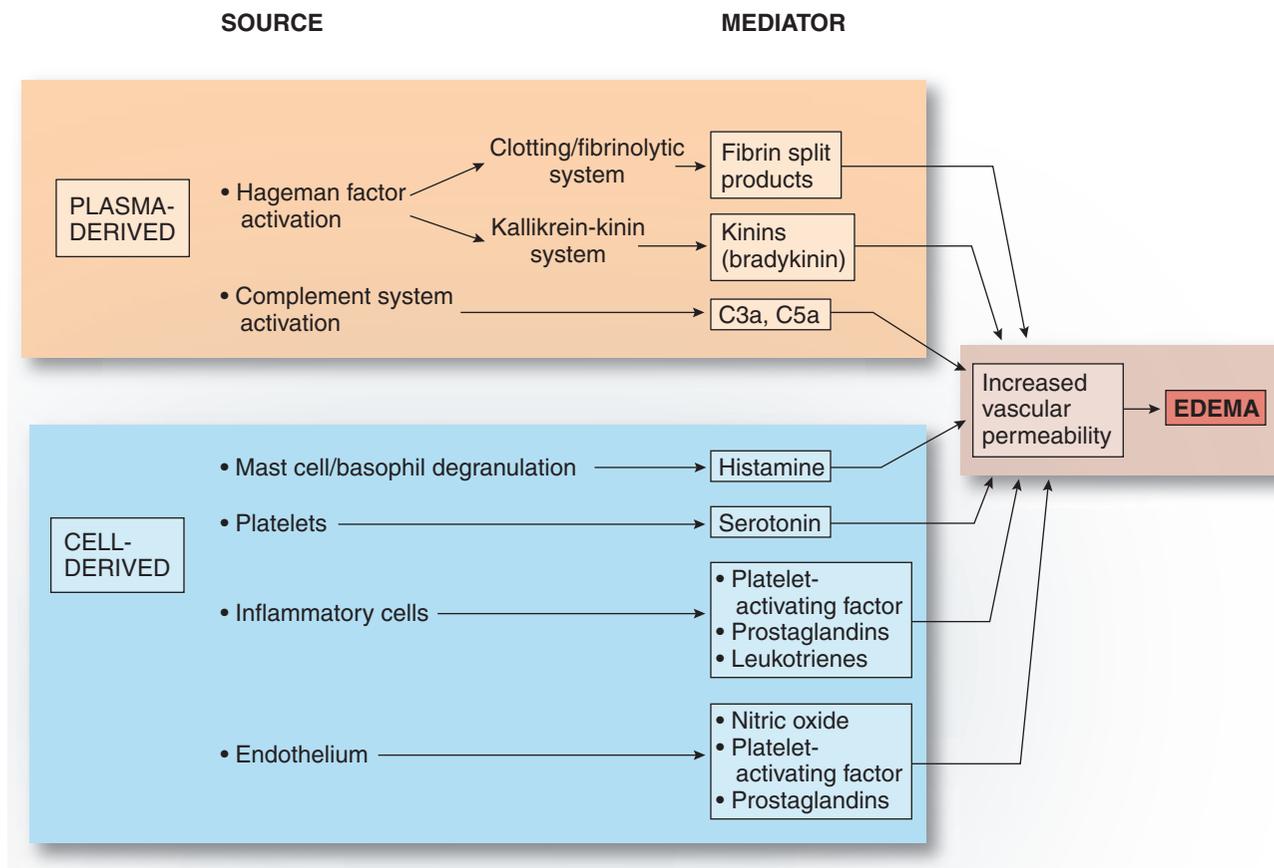
SOURCE   MEDIATOR

FIGURE 2-5. **Inflammatory mediators of increased vascular permeability.**

Vascular and Tissue Fluids Are Regulated by a Balance of Forces

Under normal circumstances, there is continual movement of fluid from the intravascular compartment to the extravascular space. Fluid that accumulates in the extravascular space is then cleared through lymphatics and returned to the circulation. Regulation of fluid transport across vascular walls is described in part by the **Starling principle**. According to this law, interchange of fluid between vascular and extravascular compartments results from a balance of forces that draw fluid into the vascular space or out into tissues (see also Chapter 7). These forces include:

- **Hydrostatic pressure** results from blood flow and plasma volume. When increased, hydrostatic pressure forces fluid out of the vasculature.

- **Oncotic pressure** reflects the plasma protein concentration, and draws fluid into vessels.

- **Osmotic pressure** is determined by relative amounts of sodium and water in vascular and tissue spaces.

- **Lymph flow**, the passage of fluid through the lymphatic system, continuously drains fluid out of tissues and into lymphatic spaces.

Noninflammatory Edema

When the balance of forces that regulate fluid transport is altered, flow into the extravascular compartment or clearance through lymphatics is disrupted. The net result is fluid accumulation in the interstitial spaces (**edema**). This excess fluid expands the spaces between cells and ECM elements, and leads to tissue swelling. A range of clinical conditions, either systemic or organ specific, are associated with edema. Obstruction of venous outflow (**thrombosis**) or decreased right ventricular function (congestive heart failure) cause back pressure in the vasculature, thereby increasing hydrostatic pressure (see Chapter 7). Loss of albumin (kidney disorders) or decreased synthesis of plasma proteins (liver disease, malnutrition) reduce plasma oncotic pressure. Any abnormality of sodium or water retention will alter the osmotic pressure and the balance of fluid forces. Finally, obstruction of lymphatic flow may occur in various clinical settings but is most commonly due to surgical removal of lymph nodes or tumor obstruction. This fluid accumulation is referred to as **lymphedema**.

Inflammatory Edema

Among the earliest responses to tissue injury are alterations in the anatomy and function of the microvasculature, which may promote fluid accumulation in tissues (see Figs. 2-4 and 2-5). These pathological changes are characteristic of the classic "triple response" first described by Sir Thomas Lewis in 1924. In the original experiments a dull red line developed at the site of mild trauma to skin, followed by a **flare** (red halo), then a **wheal** (swelling). Lewis postulated the presence of a vasoactive mediator that caused vasodilation and increased vascular permeability at the site of injury. The triple response can be explained as follows:

1. **Transient vasoconstriction of arterioles** at the site of injury is the earliest vascular response to mild skin injury. This process is mediated by both neurogenic and chemical mediator systems and usually resolves within seconds to minutes.

2. **Vasodilation of precapillary arterioles** then increases blood flow to the tissue, a condition known as **hyperemia**. Vasodilation is caused by release of specific mediators and is responsible for redness and warmth at sites of tissue injury.

3. **An increase in endothelial cell barrier permeability** results in edema. Loss of fluid from intravascular compartments as blood passes through capillary venules leads to local stasis and plugging of dilated small vessels with erythrocytes. These changes are reversible following mild injury: within several minutes to hours, the extravascular fluid is cleared through lymphatics.

The vascular response to injury is a dynamic event that involves sequential physiological and pathological changes. **Vasoactive mediators,** originating from both plasma and cellular sources, are generated at sites of tissue injury (see Fig. 2-5). These mediators bind to specific receptors on vascular endothelial and smooth muscle cells, causing vasoconstriction or vasodilation. Vasodilation of arterioles increases blood flow and can exacerbate fluid leakage into the tissue. Vasoconstriction of postcapillary venules increases capillary bed hydrostatic pressure, potentiating edema formation. Vasodilation of venules decreases capillary hydrostatic pressure and inhibits movement of fluid into extravascular spaces.

After injury, vasoactive mediators bind specific receptors on endothelial cells, causing endothelial cell contraction and gap formation, a reversible process (see Fig. 2-4B). This break in the endothelial barrier leads to extravasation (leakage) of intravascular fluids into the extravascular space. Mild direct injury to the endothelium results in a biphasic response: an early change in permeability occurs within 30 minutes after injury, followed by a second increase in vascular permeability after 3 to 5 hours. When damage is severe, exudation of intravascular fluid into the extravascular compartment increases progressively peaking 3 to 4 hours after injury.

Severe direct injury to the endothelium, such as is caused by burns or caustic chemicals, may result in irreversible damage. In such cases, the endothelium separates from the basement membrane, resulting in cell blebbing (blisters or bubbles between the endothelium and the basement membrane). This leaves areas of basement membrane naked (see Fig. 2-4C), disrupting the barrier between the intravascular and extravascular spaces.

Several definitions are important for understanding the consequences of inflammation:

- **Edema** is accumulation of fluid within the extravascular compartment and interstitial tissues.
- An **effusion** is excess fluid in body cavities, e.g., peritoneum or pleura.
- A **transudate** is edema fluid with low protein content (specific gravity < 1.015).
- An **exudate** is edema fluid with a high protein concentration (specific gravity > 1.015), which frequently contains inflammatory cells. Exudates are observed early in acute inflammatory reactions and are produced by mild injuries, such as sunburn or traumatic blisters.
- A **serous exudate,** or **effusion,** is characterized by the absence of a prominent cellular response and has a yellow, straw-like color.
- **Serosanguineous** refers to a serous exudate, or effusion, that contains red blood cells and has a red tinge.
- A **fibrinous exudate** contains large amounts of fibrin as a result of activation of the coagulation system. When a fibrinous exudate occurs on a serosal surface, such as the pleura or

FIGURE 2-6. **Fibrinous pericarditis.** The heart from a patient who died in renal failure and uremia exhibits a shaggy, fibrinous exudate covering the entire visceral pericardium.

pericardium, it is referred to as "fibrinous pleuritis" or "fibrinous pericarditis" (Fig. 2-6).

- **A purulent exudate or effusion** is one that contains prominent cellular components. Purulent exudates and effusions are frequently associated with pathological conditions such as pyogenic bacterial infections, in which the predominant cell type is the polymorphonuclear neutrophil (PMN) (Fig. 2-7).
- **Suppurative inflammation** describes a condition in which a purulent exudate is accompanied by significant liquefactive necrosis; it is the equivalent of pus.
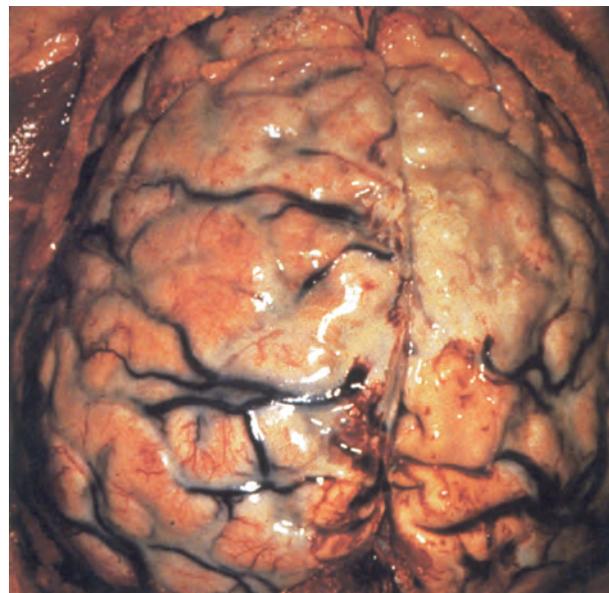
FIGURE 2-7. **Purulent exudate.** In this patient with bacterial meningitis, a viscid, cream-colored, acute inflammatory exudate is present within the subarachnoid space.

Plasma-Derived Mediators Of Inflammation

A large number of chemical mediators are integral to initiation, amplification and termination of inflammatory processes (Fig. 2-8). Cell- and plasma-derived mediators work in concert to activate cells by binding specific receptors, activating cells, recruiting cells to sites of injury, and stimulating of release of additional soluble mediators. These mediators themselves are relatively short-lived, or are inhibited by intrinsic mechanisms, effectively turning off the response and allowing the process to resolve. These are thus important "on" and "off" control mechanisms of inflammation. Cell-derived mediators are considered below.

Plasma contains the elements of three major enzyme cascades, each composed of a series of proteases. Sequential activation of proteases results in release of important chemical mediators. These interrelated systems include (1) the **coagulation cascade**, (2) **kinin generation**, and (3) the **complement system** (Fig. 2-9). The coagulation cascade is discussed in Chapters 10 and 20; the kinin and complement systems are presented here.

## Hageman Factor is a Key Source of Vasoactive Mediators

Hageman factor (clotting factor XII), generated within the plasma, is activated by exposure to negatively charged surfaces such as basement membranes, proteolytic enzymes, bacterial lipopolysaccharide, and foreign materials. This key component triggers activation of additional plasma proteases leading to:
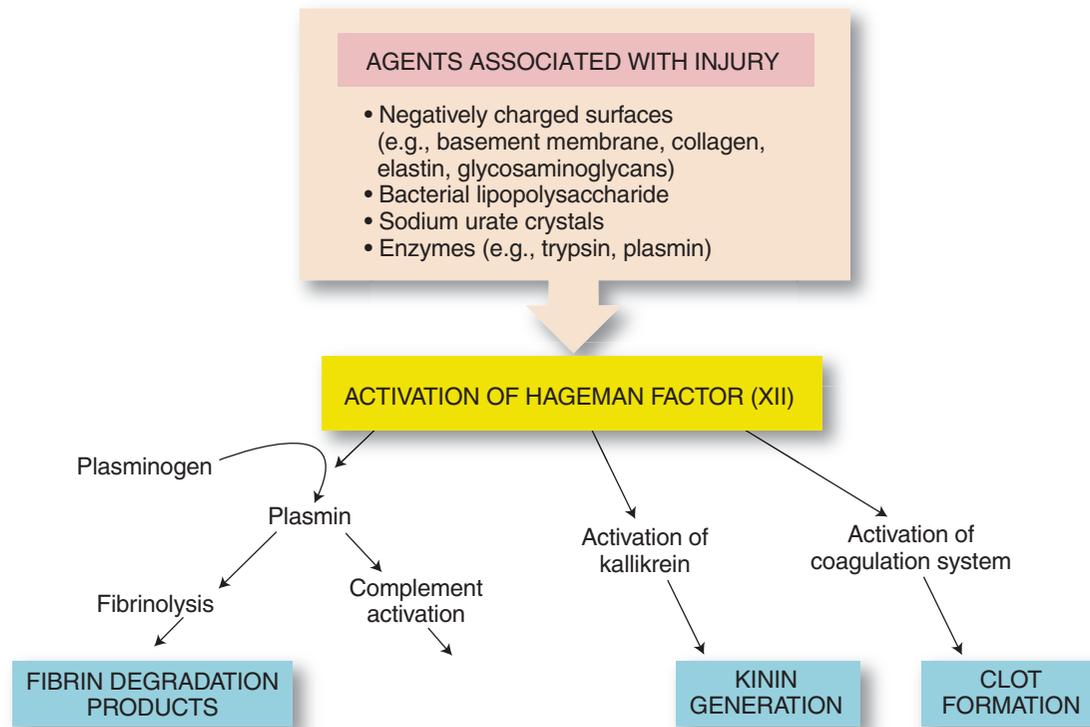
- **Conversion of plasminogen to plasmin:** Plasmin generated by activated Hageman factor induces fibrinolysis. Products of fibrin degradation (fibrin-split products) augment vascular permeability in the skin and the lung. Plasmin also cleaves components of the complement system, generating biologically active products, including the anaphylatoxins C3a and C5a.
- **Conversion of prekallikrein to kallikrein:** Plasma kallikrein, also generated by activated Hageman factor, cleaves high-molecular-weight kininogen, thereby producing several vasoactive low molecular weight peptides, collectively termed **kinins**.
- **Activation of the alternative complement pathway.**
- **Activation of the coagulation system** (see Chapters 10 and 20).

Kinins Amplify the Inflammatory Response

Kinins are potent inflammatory agents formed in plasma and tissue by the action of serine protease kallikreins on specific plasma glycoproteins termed **kininogens**. **Bradykinin** and related peptides regulate multiple physiological processes including blood pressure, contraction and relaxation of smooth muscle, plasma
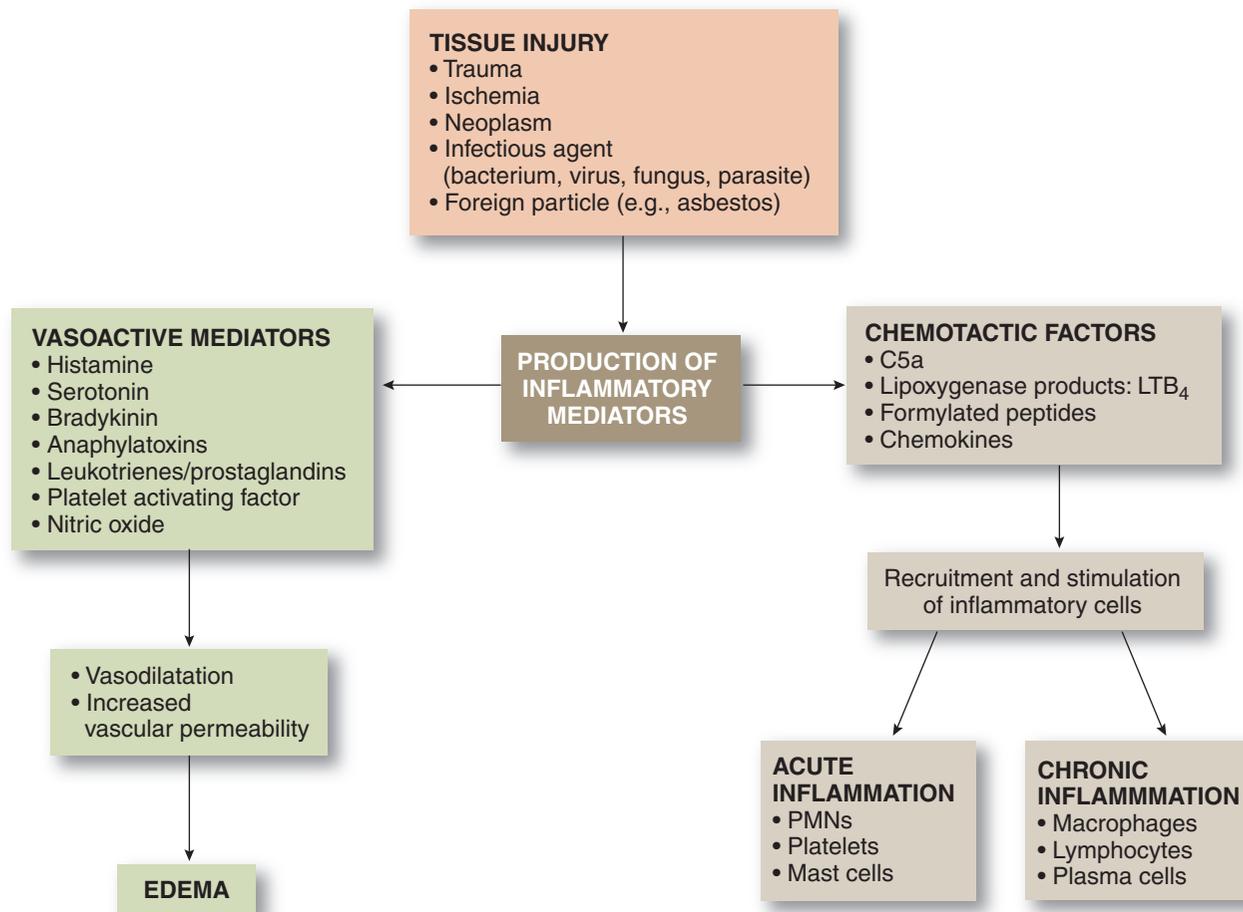
FIGURE 2-8. **Mediators of the inflammatory response.** Tissue injury stimulates the production of inflammatory mediators in plasma and released in the circulation. Additional factors are generated by tissue cells and inflammatory cells. These vasoactive and chemotactic mediators promote edema and recruit inflammatory cells to the site of injury. PMNs = polymorphonuclear neutrophils.

FIGURE 2-9. **Hageman factor activation and inflammatory mediator production.** Hageman factor activation is a key event leading to conversion of plasminogen to plasmin, resulting in generation of fibrin split products and active complement products. Activation of kallikrein produces kinins and activation of the coagulation system results in clot formation.

extravasation, cell migration, inflammatory cell activation, and inflammatory-mediated pain responses. The immediate effects of kinins are mediated by two receptors: B_1 receptors are induced by inflammatory mediators and are selectively activated by bradykinin metabolites, and B_2 receptors are constitutively and widely expressed. Kinins are rapidly degraded to inactive products by kininases and therefore have rapid and short-lived functions. Perhaps the most significant function of kinins is their ability to amplify the inflammatory response by stimulating local tissue cells and inflammatory cells to generate additional mediators, including prostanoids, cytokines (especially tumor necrosis factor-α [TNF-α] and interleukins), nitric oxide, and tachykinins.

Complement Is Activated through Three Pathways To Form The Membrane Attack Complex

The complement system is a group of proteins found in plasma and on cell surfaces, whose primary function is defense against microbes. First identified as a heat-labile serum factor that kills bacteria and "complements" antibodies, the complement system consists of more than 30 proteins—including plasma enzymes, regulatory proteins, and cell lysis proteins—whose principal site of synthesis is the liver. The physiological activities of the complement system include (1) defense against pyogenic bacterial infection by opsonization, chemotaxis, activation of leukocytes and lysis of bacteria and cells; (2) bridging innate and adaptive immunity for defense against microbial agents by augmenting antibody responses and enhancing immunological memory; and (3) disposal of immune products and products of inflammatory injury by clearance of immune complexes from tissues and removal of apoptotic cells. Certain complement components, **anaphylatoxins,** are vasoactive mediators. Other components fix opsonins on

cell surfaces and still others induce cell lysis by generating the lytic complex C5b-9 (**membrane attack complex [MAC]**). The proteins involved in activating the complement system are themselves activated by three convergent pathways termed **classical, mannose-binding lectin (MBL),** and **alternative**.

The Classical Pathway

Activators of the classical pathway include antigen-antibody (Ag-Ab) complexes, products of bacteria and viruses, proteases, urate crystals, apoptotic cells, and polyanions (polynucleotides). The proteins of this pathway are C1 through C9, the nomenclature following the historical order of discovery. Ag-Ab complexes activate C1, initiating a cascade that leads to formation of the MAC proceeds as follows (Fig. 2-10):

1. **Antibodies bound to antigens on bacterial cell surfaces bind the C1 complex.** The C1 complex consists of C1q, two molecules of C1r, and two molecules of C1s. Antibodies in the immune complexes bind C1q, thereby triggering activation of C1r and C1s.

2. **C1s first cleaves C4, which binds the bacterial surface and then cleaves C2.** The resulting cleaved molecules form the C4b2a enzyme complex, also called **C3 convertase,** which remains covalently bound to the bacterial surface. This anchors the complement system at specific tissue sites. If a covalent bond is not formed, the complex is inactivated, aborting the complement cascade in normal host cells or tissues.

3. **C3 convertase cleaves C3 into C3a and C3b.** This is a critical step in generating biologically active complement components. C3a is released as an **anaphylatoxin**. C3b reacts with cell proteins to localize, or "fix" on the cell surface. C3b and its degradation products, especially iC3b, on the surface
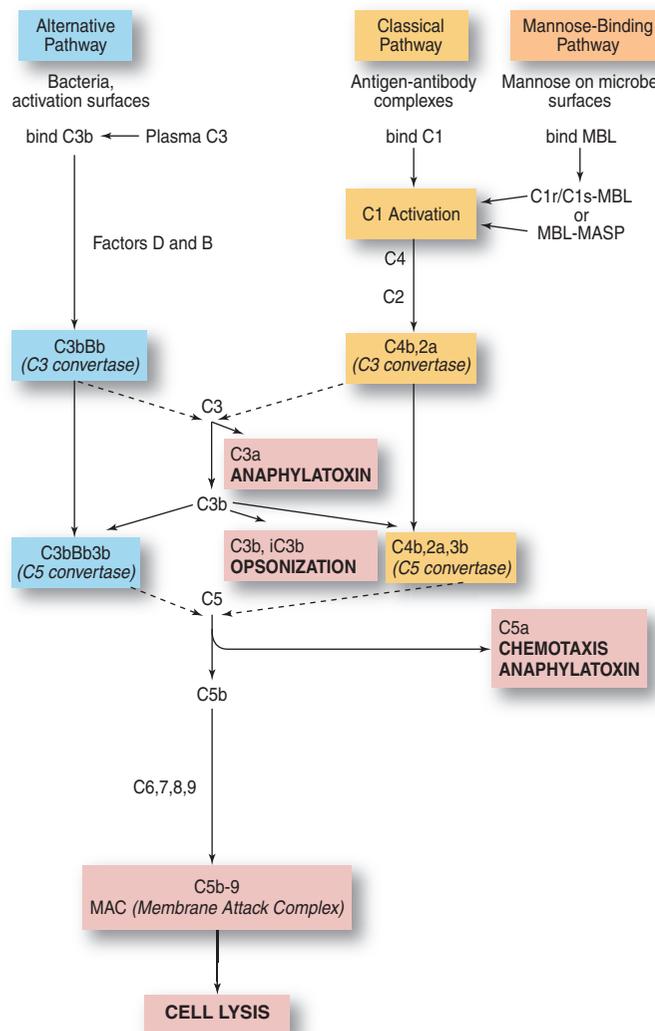
FIGURE 2-10. Complement activation. The alternative, classical, and mannose-binding pathways lead to generation of the complement cascade of inflammatory mediators and cell lysis by the membrane attack complex (MAC). MBL = mannose-binding lectin; MBL-MASP = MBL-associated serine protease.

of pathogens, enhance phagocytosis. This process of coating a pathogen with a molecule that enhances phagocytosis is **opsonization**, and the molecule is referred to as an **opsonin**.

4. **The complex of C4b, C2a and C3b (termed C5 convertase) cleaves C5 into C5a and C5b.** C5a also is an anaphylatoxin, and C5b acts as the nidus for the sequential binding of C6, C7, and C8 to form the MAC.

5. **The MAC assembles on target cells**. The MAC directly inserts into the plasma membrane by hydrophobic binding of C7 to the lipid bilayer. The resulting cylindrical transmembrane channel disrupts the barrier function of the plasma membrane and leads to cell lysis.

The Mannose-Binding Pathway

The mannose- or lectin-binding pathway has some components in common with the classical pathway. It is initiated by binding of microbes bearing terminal mannose groups to **MBL**, a member of the family of calcium-dependent lectins, termed the **collectins**. This multifunctional acute phase protein has properties similar to those of immunoglobulin (Ig) M (IgM) antibody (it binds to a wide range of oligosaccharide structures), IgG (it

interacts with phagocytic receptors) and C1q. This last property enables it to interact with either C1r-C1s or with a serine protease called MASP (MBL-associated serine protease) to activate complement as follows (see Fig. 2-10):

1. **MBL interacts with C1r and C1s to generate C1 esterase activity.** Alternatively and preferentially, MBL forms a complex with a precursor of the serine protease MASP. MBL and MASP bind to mannose groups on glycoproteins or carbohydrates on bacterial cell surfaces. After MBL binds a substrate, the MASP proenzyme is cleaved into two chains and expresses a C1-esterase activity.

2. **C1-esterase activity, either from C1r/C1s- MBL interaction or MBL-MASP, cleaves C4 and C2, leading to assembly of the classical pathway C3 convertase.** The complement cascade then continues as described for the classical pathway.

Alternative Pathway

The alternative pathway is initiated by derivative products of microorganisms such as endotoxin (from bacterial cell surfaces), zymosan (yeast cell walls), polysaccharides, cobra venom factor, viruses, tumor cells, and foreign materials. Proteins of the alternative pathway are called "factors," followed by a letter. Activation of the alternative pathway proceeds as follows (see Fig. 2-10):

1. **A small amount of C3 in plasma cleaves to C3a and C3b.** This C3b is covalently bound to carbohydrates and proteins on microbial cell surfaces. It binds factor B and factor D to form the alternative pathway C3 convertase, C3bBb. This C3 convertase is stabilized by **properdin**.

2. **C3 convertase generates additional C3b and C3a.** Binding of a second C3b molecule to the C3 convertase converts it to a C5 convertase, C3bBb3b.

3. **As in the classical pathway, cleavage of C5 by C5 convertase generates C5b and C5a and leads to assembly of the MAC.**

The Complement System is Tightly Regulated to Generate Proinflammatory Molecules

Biological Activities of Complement Components

The endpoint of complement activation is formation of the MAC and cell lysis. The cleavage products generated at each step of the way both catalyze the next step in the cascade, and themselves have additional properties that render them important inflammatory molecules:

- **Anaphylatoxins** (C3a, C4a, C5a): These proinflammatory molecules mediate smooth muscle contraction and increase vascular permeability (Fig. 2-11).

- **Opsonins** (C3b, iC3b): Bacterial opsonization is the process by which a specific molecule (e.g., IgG or C3b) binds to the surface of the bacterium. The process enhances phagocytosis by enabling receptors on phagocytic cell membranes (e.g., Fc receptor or C3b receptor) to recognize and bind the opsonized bacterium. Viruses, parasites, and transformed cells also activate complement by similar mechanisms, an effect that leads to their inactivation or death.

- **Proinflammatory molecules** (MAC, C5a): These chemotactic factors also activate leukocytes and tissue cells to generate oxidants and cytokines, induce degranulation of mast cells and basophils.
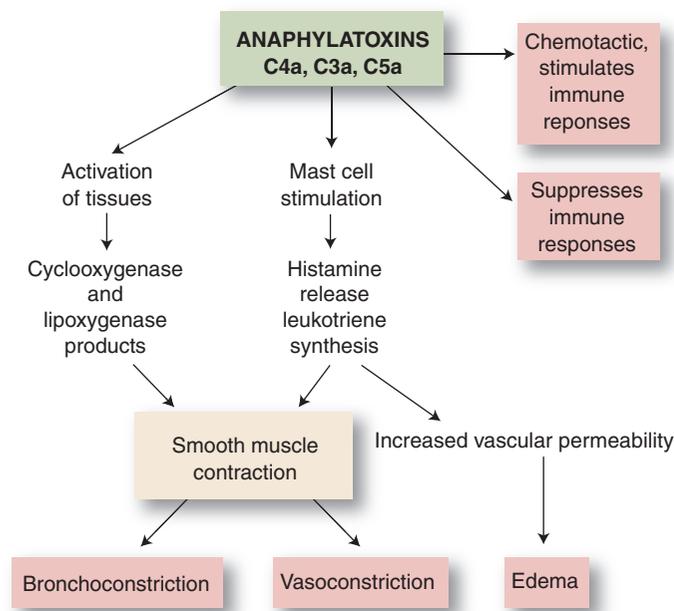
FIGURE 2-11. Biological activity of the anaphylatoxins. Complement activation products, generated during activation of the complement cascade, regulate vascular permeability, cell recruitment, and smooth muscle contraction.

- **Lysis (MAC):** C5b binds C6 and C7, and subsequently C8 to the target cell; C9 polymerization is catalyzed to lyse the cell membrane.

Regulation of the Complement System

Proteins in serum and on cell surfaces protect the host from indiscriminate injury by regulating complement activation. There are 4 major mechanisms for this:

- **Spontaneous decay:** C4b2a and C3bBb and their cleavage products, C3b and C4b, decrease by decay.
- **Proteolytic inactivation:** Plasma inhibitors include factor 1 (an inhibitor of C3b and C4b) and serum carboxypeptidase N (SCPN). SCPN cleaves the carboxy-terminal arginine from anaphylatoxins C4a, C3a, and C5a. Removing this single amino acid markedly decreases the biological activity of each of these molecules.
- **Binding of active components:** C1 esterase inhibitor (C1 INA) binds C1r and C1s, forming an irreversibly inactive complex. Additional binding proteins in the plasma include factor H and C4b binding protein. These proteins complex with C3b and C4b, respectively, and enhance their susceptibility to proteolytic cleavage by factor I.
- **Cell membrane-associated molecules:** Two proteins linked to the cell membrane by glycophosphoinositol (GPI) anchors are decay-accelerating factor (DAF) and protectin (CD59). DAF breaks down the alternative pathway C3 convertase, CD59 (membrane cofactor protein, protectin) binds membrane-associated C4b and C3b, promotes its inactivation by factor I and prevents formation of the MAC.

The Complement System and Disease

The complement system is exquisitely regulated so that activation of complement is focused on the surfaces of microorganisms, whereas deposition on normal cells and tissues is limited. When the mecha-nisms regulating this balance do not function properly, or are deficient because of mutation, resulting imbalances in complement activity can cause tissue injury (Table 2-1).

Immune Complexes

Immune complexes (Ag-Ab complexes) form on bacterial surfaces and associate with C1q, activating the classical pathway. Complement then promotes the physiological clearance of circulating immune complexes. However, when these complexes are formed continuously and in excess (e.g., in chronic immune responses), the relentless activation of complement results in its consumption and, therefore, net depletion of complement. Complement inefficiency, whether due to complement depletion, deficient complement binding, or defects in complement activation, results in immune deposition and inflammation, which in turn may trigger autoimmunity.

Infectious Disease

Defense against infection is a key role of complement products, and defective functioning of the complement system leads to increased susceptibility to infection. C3b and iC3b, the cleavage fragments of C3, normally bind bacterial surfaces to promote phagocytosis of the bacteria. Increased susceptibility to pyogenic infection by organisms such as *Haemophilus influenzae* and *Streptococcus pneumoniae* is associated with defects in antibody production, complement proteins, or phagocyte function. Deficiencies in formation of MAC are associated with increased infections, particularly with meningococci. Deficiency of complement MBL results in recurrent infections in young children. For some bacteria, thick bacterial capsules can prevent lysis by complement. Furthermore bacterial enzymes can inhibit the effects of complement components, especially C5a, or increase catabolism of components, such as C3b, thereby reducing formation of C3 convertase. Viruses, on the other hand, may use cell-bound components and receptors to facilitate cell entry. *Mycobacterium tuberculosis,* Epstein-Barr virus, measles virus, picornaviruses, human immunodeficiency virus (HIV), and flaviviruses use complement components to target inflammatory or epithelial cells.

Inflammation and Necrosis

The complement system amplifies the inflammatory response. Anaphylatoxins C5a and C3a activate leukocytes, and C5a and MAC

TABLE 2–1	
Hereditary Complement Deficiencies	
Complement Deficiency	**Clinical Association**
C3b, iC3b, C5, MBL	Pyogenic bacterial infections
	Membranoproliferative glomerulonephritis
C3, properdin, MAC proteins	Neisserial infection
C1 Inhibitor	Hereditary angioedema
CD59	Hemolysis, thrombosis
C1q, C1r and C1s, C4, C2	Systemic lupus erythematosus
Factor H and Factor I	Hemolytic-uremic syndrome Membranoproliferative glomerulonephritis

MAC = membrane attack complex; MBL = mannose-binding lectin.

activate endothelial cells, inducing generation of oxidants and cytokines that are harmful to tissues when in excess (see Chapter 1). Nonviable or injured tissues cannot regulate complement normally.

Complement Deficiencies

The importance of an intact and appropriately regulated complement system is exemplified in persons who have acquired or congenital deficiencies of specific complement components or regulatory proteins (see Table 2-1). The most common congenital defect is a C2 deficiency, inherited as an autosomal codominant trait. Acquired deficiencies of early complement components occur in patients with some autoimmune diseases, especially those associated with circulating immune complexes. These include certain forms of membranous glomerulonephritis and systemic lupus erythematosus. Deficiencies in early components of complement, e.g., C1q, C1r, C1s, and C4, are strongly associated with susceptibility to systemic lupus erythematosus; patients lacking the middle (C3, C5) components are prone to recurrent pyogenic infections, membranoproliferative glomerulonephritis and rashes; those who lack terminal complement components (C6, C7, or C8) are vulnerable to infections with *Neisseria* species. Such differences in susceptibility further emphasize the importance of individual complement components in host surveillance against bacterial infection. Congenital defects in proteins that regulate the complement system, e.g., C1 inhibitor and SCPN, result in chronic complement activation. C1 inhibitor deficiency is also associated with the syndrome of hereditary angioedema.

## Cell-Derived Mediators of Inflammation

Circulating platelets, basophils, PMNs, endothelial cells monocyte/macrophages, tissue mast cells, and the injured tissue itself are all potential cellular sources of vasoactive mediators. In general, these mediators are (1) derived from metabolism of phospholipids and arachidonic acid (e.g., prostaglandins, thromboxanes, leukotrienes, lipoxins, platelet-activating factor [PAF]), (2) preformed and stored in cytoplasmic granules (e.g., histamine, serotonin, lysosomal hydrolases), or (3) derived from altered production of normal regulators of vascular function (e.g., nitric oxide and neurokinins).

Arachidonic Acid and Platelet-Activating Factor Are Derived from Membrane Phospholipids

Phospholipids and fatty acid derivatives released from plasma membranes are metabolized into mediators and homeostatic regulators by inflammatory cells and injured tissues (Fig. 2-12). As part of a complex regulatory network, prostanoids, leukotrienes, and lipoxins, which are derivatives of arachidonic acid, both promote and inhibit inflammation (Table 2-2). The impact depends on several factors, including the level and profile of prostanoid production, both of which change during an inflammatory response.

Arachidonic Acid

Depending on the specific inflammatory cell and the nature of the stimulus, activated cells generate arachidonic acid by one of two pathways (see Fig 2-12). One pathway involves liberation of arachidonic acid from the glycerol backbone of cell membrane phospholipids (in particular, phosphatidylcholine) by stimulus-
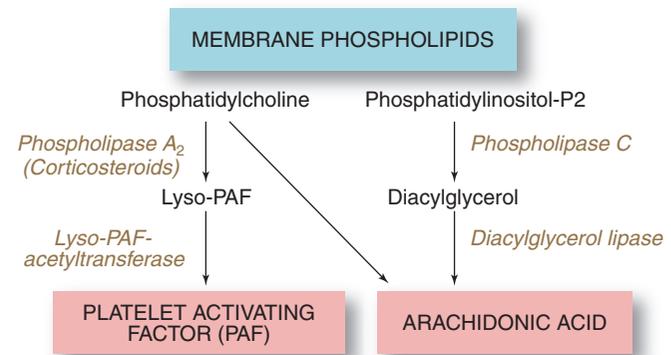
FIGURE 2-12. Cell membrane-derived mediators. Platelet activating factor (PAF) is derived from choline-containing glycerophospholipids in the membrane. Arachidonic acid derives from phosphatidylinositol phosphates and from phosphatidyl choline.

induced activation of phospholipase A_2 (PLA_2). The other is metabolism of phosphatidylinositol phosphates to diacylglycerol and inositol phosphates by phospholipase C. Diacylglycerol lipase then cleaves arachidonic acid from diacylglycerol. Once generated, arachidonic acid is further metabolized through two pathways: (1) **cyclooxygenation**, with subsequent production of prostaglandins and thromboxanes; and (2) **lipoxygenation**, to form leukotrienes and lipoxins (Fig. 2-13).

Corticosteroids are widely used to suppress tissue destruction associated with many inflammatory diseases, including allergic responses, rheumatoid arthritis, and systemic lupus erythematosus. Corticosteroids induce synthesis of an inhibitor of PLA_2 and block release of arachidonic acid in inflammatory cells. Although corticosteroids (e.g., prednisone) are widely used to suppress inflammatory responses, their prolonged administration can have significant harmful effects, including increased risk of infection, damage to connective tissue, and adrenal gland atrophy.

TABLE 2–2	
Biological Activities of Arachidonic Acid Metabolites	
Metabolite	**Biological Activity**
PGE_2, PDG_2	Induce vasodilation, bronchodilation; inhibit inflammatory cell function
PGI_2	Induces vasodilation, bronchodilation; inhibits inflammatory cell function
$PGF_{2\alpha}$	Induces vasodilation, bronchoconstriction
TXA_2	Induces vasoconstriction, bronchoconstriction; enhances inflammatory cell functions (esp. platelets
LTB_4	Chemotactic for phagocytic cells; stimulates phagocytic cell adherence; enhances microvascular permeability
LTC_4, LTD_4, LTE_4	Induce smooth muscle contraction; constrict pulmonary airways; increase microvascular permeability

PG . . . = prostaglandin; PD . . . = ; LT . . . = leukotriene; TXA_2 = thromboxane A2
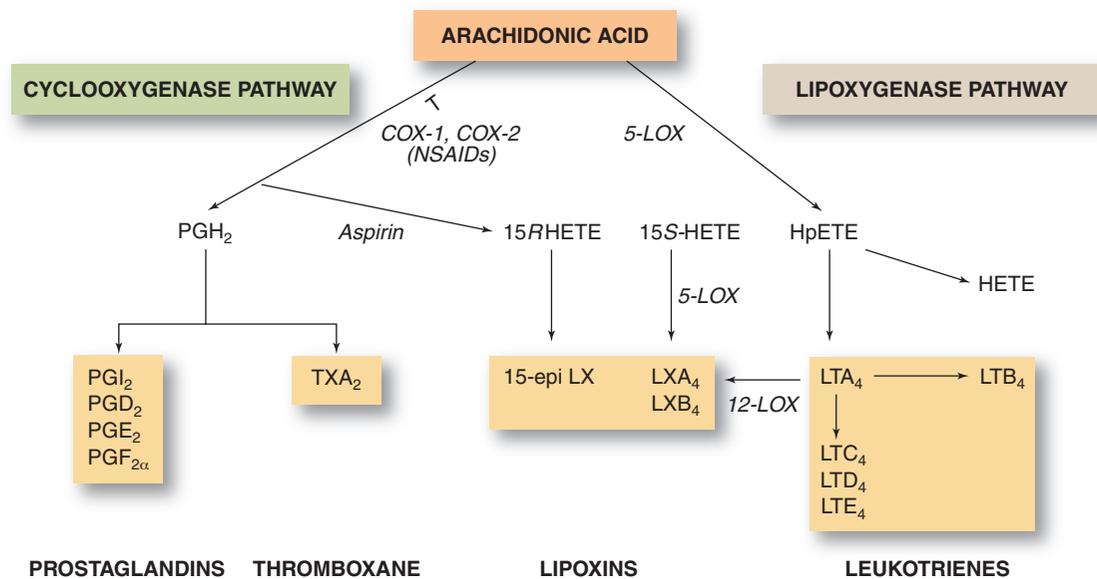
FIGURE 2-13. **Biologically active arachidonic acid metabolites.** The cyclo-oxygenase pathway of arachidonic acid metabolism generates prostaglandins (PG. . .) and thromboxane (TXA_2). The lipoxygenase pathway forms lipoxins (LX. . .) and leukotrienes (LT. . .); COX = cyclooxygenase; HETE = hydroxyicosatetraenoic acid ; HpETE = 5-hydroperoxyeicosatetraenoic acid; NSAIDs = nonsteroidal anti-inflammatory drugs.

Platelet-Activating Factor

Another potent inflammatory mediator derived from membrane phospholipids is PAF, synthesized by virtually all activated inflammatory cells, endothelial cells, and injured tissue cells. During inflammatory and allergic responses, PAF is derived from choline-containing glycerophospholipids in the cell membrane, initially by PLA_2, followed by acetylation by an acetyltransferase (see Fig. 2-12). In plasma, PAF-acetylhydrolase controls PAF activity. PAF has a wide range of activities. PAF stimulates platelets, neutrophils, monocyte/macrophages, endothelial cells, and vascular smooth muscle cells. PAF-induced platelet aggregation and degranulation at sites of tissue injury and enhances release of serotonin, thereby causing changes in vascular permeability. Because PAF primes leukocytes, it enhances functional responses (e.g., O_2 production, degranulation) to a second stimulus and induces adhesion molecule expression, specifically of integrins. PAF is also an extremely potent vasodilator, augmenting permeability of microvasculature at sites of tissue injury. PAF generated by endothelial cells cooperates with P-selectin. When P-selectin lightly tethers a leukocyte to an endothelial cell, PAF from the endothelial cell binds its receptor on the leukocyte and induces intracellular signaling.

Prostanoids, Leukotrienes, and Lipoxins Are Biologically Active Metabolites Of Arachidonic Acid

Prostanoids

Arachidonic acid is further metabolized by cyclooxygenases 1 and 2 (COX-1, COX-2) to generate prostanoids (see Fig. 2-13). **COX-1** is constitutively expressed by most cells and increases upon cell activation. It is a key enzyme in the synthesis of prostaglandins, which in turn (1) protect the gastrointestinal mucosal lining, (2) regulate water/electrolyte balance, (3) stimulate platelet aggregation to maintain normal hemostasis, and (4) maintain resistance to thrombosis on vascular endothelial cell surfaces. **COX-2** expression is generally low or undetectable, but

increases substantially upon stimulation, generating metabolites important in inducing pain and inflammation.

The early inflammatory prostanoid response is COX-1-dependent; COX-2 takes over as the major source of prostanoids as inflammation progresses. Both COX isoforms generate prostaglandin H (PGH_2), which is then the substrate for production of prostacyclin (PGI_2), PGD_2, PGE_2, $PGF_{2\alpha}$, and TXA_2 (thromboxane). The profile of prostaglandin production (i.e., the quantity and variety produced during inflammation) depends in part on the cells present and their activation state. Thus, mast cells produce predominantly PGD_2; macrophages generate PGE_2 and TXA_2; platelets are the major source of TXA_2; endothelial cells produce PGI_2. Prostanoids affect immune cell function by binding G protein-coupled cell surface receptors, leading to activation of a range of intracellular signaling pathways in immune cells and resident tissue cells. The repertoire of prostanoid receptors expressed by various immune cells differs, so the functional responses of these cells may be modified differently according to the prostanoids present.

Inhibition of COX is one mechanism by which non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, indomethacin, and ibuprofen, exert their potent analgesic and antiinflammatory effects. NSAIDS block COX-2–induced formation of prostaglandins, thereby mitigating pain and inflammation. However, they also affect COX-1, lead to decreased homeostatic functions, and so affect the stomach and kidneys adversely. This complication led to development of COX-2–specific inhibitors.

Leukotrienes

Slow-reacting substance of anaphylaxis (SRS-A) has long been recognized as a smooth muscle stimulant and mediator of hypersensitivity reactions. It is, in fact, a mixture of leukotrienes, the second major family of derivatives of arachidonic acid (see Fig. 2-13). The enzyme 5-lipoxygenase (5-LOX) leads to synthesis of 5-hydroperoxyeicosatetraenoic acid (5-HpETE) and leukotriene A_4 (LTA_4) from arachidonic acid; the latter is a precursor for other leukotrienes. In neutrophils and certain

macrophage populations, LTA_4 is metabolized to LTB_4, which has potent chemotactic activity for neutrophils, monocytes, and macrophages. In other cell types, especially mast cells, basophils, and macrophages, LTA_4 is converted to LTC_4 and thence to LTD_4 and LTE_4. These three cysteinyl-leukotrienes: (1) stimulate smooth muscle contraction, (2) enhance vascular permeability, and (3) are responsible for development of many of the clinical symptoms associated with allergic-type reactions. They thus play a pivotal role in the development of asthma. Leukotrienes exert their action through high-affinity specific receptors that may prove to be important targets of drug therapy.

Lipoxins

Lipoxins, the third class of products of arachidonic acid, are made within the vascular lumen by cell–cell interactions (see Fig. 2-13). They are proinflammatory, trihydroxytetraene-containing eicosanoids that are generated during inflammation, atherosclerosis, and thrombosis. Several cell types synthesize lipoxins from leukotrienes. LTA_4, released by activated leukocytes, is available for transcellular enzymatic conversion by neighboring cell types. When platelets adhere to neutrophils, LTA_4 from neutrophils is converted by platelet 12-lipoxygenase, forming lipoxin A_4 and B_4 (LXA_4 and LXB_4). Monocytes, eosinophils, and airway epithelial cells generate 15S-hydroxyeicosatetraenoic acid (15S-HETE), which is taken up by neutrophils and converted to lipoxins via 5-LOX. Activation of this pathway can also inhibit leukotriene biosynthesis, thereby providing a regulatory pathway.

Aspirin initiates transcellular biosynthesis of a group of lipoxins termed "aspirin-triggered lipoxins," or 15-epi-lipoxins (15-epi-LXs). When aspirin is administered in the presence of inflammatory mediators, 15R-HETE is generated by COX-2. Activated neutrophils convert 15R-HETE to 15 epimeric lipoxins (15-epi-LXs), which are anti-inflammatory lipid mediators. This is another pathway for the beneficial effects of aspirin.

Cytokines Are Cell-Derived Inflammatory Hormones

Cytokines constitute a group of low-molecular-weight proteins secreted by cells. Many cytokines are produced at sites of inflammation, including interleukins, growth factors, colony stimulating factors, interferons and chemokines (Fig. 2-14).
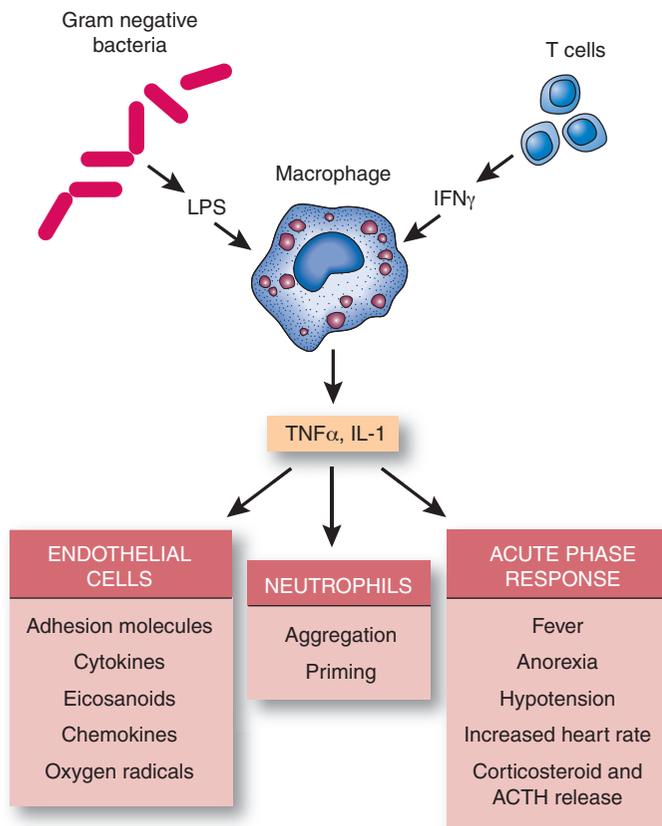
Cytokines

Cytokines produced at sites of tissue injury regulate inflammatory responses, ranging from initial changes in vascular permeability to resolution and restoration of tissue integrity. These molecules are inflammatory hormones that exhibit **autocrine** (affecting themselves), **paracrine** (affecting nearby cells), and **endocrine** (affecting cells in other tissues) functions. While most cells produce cytokines, they differ in their cytokine repertoire. *Through production of cytokines, macrophages are pivotal in orchestrating tissue inflammatory responses.* **Lipopolysaccharide** (LPS), a molecule derived from the outer cell membrane of gram-negative bacteria, is one of the most potent activators of macrophages, as well as endothelial cells and leukocytes (Fig. 2-15). LPS activates cells via specific receptors, either directly or after binding a serum LPS-binding protein (LBP). It is a potent stimulus for production of TNF-α and interleukins (IL-1, IL-6, IL-8, IL-12, and others). Macrophage-derived cytokines modulate endothelial cell–leukocyte adhesion (TNF-α), leukocyte recruitment (IL-8), the acute phase response (IL-6, IL-1), and immune functions (IL-1, IL-6, IL-12).

IL-1 and TNF-α, produced by macrophages as well as other cells, are central to development and amplification of inflammatory responses. These cytokines activate endothelial cells to express adhesion molecules and release cytokines, chemokines, and reactive oxygen species (ROS; see below). TNF-α induces priming and aggregation of neutrophils. IL-1 and TNF-α are also among the mediators of fever, catabolism of muscle, shifts in protein synthesis, and hemodynamic effects associated with inflammatory states (see Fig. 2-15).

Interleukins	Growth Factors	Chemokines	Interferons	Pro-Inflammatory Cytokines
IL-1 IL-6 IL-8 IL-13 IL-10	GM-CSF M-CSF	CC CXC XC CX3C	IFNα IFNβ IFNγ	TNFα
• Inflammatory cell activation	• Macrophage • Bactericidal activity • NK and dendritic cell function	• Leukocyte chemotaxis • Leukocyte activation	• Antiviral • Leukocyte activation	• Fever • Anorexia • Shock • Cytotoxicity • Cytokine induction • Activation of endothelial cells and tissue cells

FIGURE 2-14. **Cytokines important in inflammation.** GM-CSF = Granulocyte macrophage-colony stimulating factor; IL = interleukin; NK = natural killer; IFN = interferon; TNF = tumor necrosis factor.

FIGURE 2-15. **Central role of interleukin (IL)-1 and tumor necrosis factor (TNF)-a in inflammation.** Lipopolysaccharide (LPS) and IFN-γ activate macrophages to release inflammatory cytokines, principally IL-1 and TNF-α, responsible for directing local and systemic inflammatory responses. ACTH = adrenocorticotrophic hormone.

Interferon-gamma (IFN-γ), another potent stimulus for macrophage activation and cytokine production, is produced by a subset of T lymphocytes as part of the immune response (see Chapter 4). It is also synthesized by natural killer (NK) cells in the primary host response to intracellular pathogens (e.g., *Listeria monocytogenes*) and certain viral infections. NK cells migrate into tissues at sites of injury. When exposed to IL-12 and TNF-α, NK cells are activated to produce IFN-γ. Thus, an amplification pathway exists by which activated tissue macrophages produce TNF-α and IL-12, stimulating IFN-γ production by NK cells, with subsequent stimulation of additional macrophages.

Chemokines

Chemotactic cytokines, or chemokines, direct cell migration (**chemotaxis**). Accumulation of inflammatory cells at sites of tissue injury requires their migration from the vascular space into extravascular tissue. During migration, the cell extends a pseudopod towards increasing chemokine concentration. At the leading front of the pseudopod, marked changes in levels of intracellular calcium are associated with assembly and contraction of cytoskeleton proteins. This process draws the remaining tail of the cell along the chemical gradient. The most important chemotactic factors for PMNs are

- C5a, derived from complement
- Bacterial and mitochondrial products, particularly low-molecular-weight N-formylated peptides (such as *N*-formyl-methionyl-leucyl-phenylalanine [FMLP])

- Products of arachidonic acid metabolism, especially LTB_4
- Chemokines

Chemokines are a large class of cytokines (over 50 known members) that regulate leukocyte trafficking in inflammation and immunity. Unlike other cytokines, chemokines are small molecules that interact with G-protein–coupled receptors on target cells. These secreted proteins are produced by a variety of cell types, either constitutively or after induction, and differ widely in biological action. This diversity is based on specific cell types targeted, specific receptor activation, and differences in intracellular signaling.

Two functional classes of chemokines have been distinguished: **inflammatory chemokines** and **homing chemokines**. Inflammatory chemokines are produced in response to bacterial toxins and inflammatory cytokines (especially, IL-1, TNF-α, and IFN-γ) by a variety of tissue cells as well as leukocytes themselves. These molecules recruit leukocytes during host inflammatory responses. Homing chemokines are constitutively expressed and upregulated during disease states and direct trafficking and homing of lymphocytes and dendritic cells to lymphoid tissues during an immune response (see Chapter 4).

Structure and Nomenclature
Chemokines are synthesized as secretory proteins consisting of approximately 70 to 130 amino acids, with four conserved cysteines linked by disulfide bonds. The two major subpopulations, termed CXC or CC chemokines (formerly called α and β chemokines)–are distinguished by the position of the first two cysteines, which are either separated by one amino acid (CXC) or are adjacent (CC). Two additional classes of chemokines, each with a single member, have been identified. Lymphotactin has two instead of four conserved cysteines (XC), and fractaline (or neurotactin) has three amino acids between the first two cysteines (CX_3C). Chemokines are named according to their structure, followed by "L" and the number of their gene (CCL1, CXCL1, etc.). However, many of the traditional names for chemokines persist in current usage. Chemokine receptors are named according to their structure, "R," and a number (CCR1, CXCR1, etc.); most receptors recognize more than one chemokine and most chemokines recognize more than one receptor. Receptor binding of chemokines to their ligands may result in an agonistic or antagonistic activity. The same chemokine may act as an agonist for one receptor and an antagonist for another. Leukocyte recruitment or lymphocyte homing is modulated by a combination of these agonistic and antagonistic activities.

Anchoring and Activity
Chemokines function as immobilized or soluble molecules. They generate a chemotactic gradient by binding to proteoglycans of the ECM or to cell surfaces. As a result, high concentrations of chemokines persist at sites of tissue injury. Specific receptors on the surface of the migrating leukocytes bind the matrix-bound chemokines and associated adhesion molecules, which tends to move cells along the chemotactic gradient to the injury site. This process of responding to a matrix-bound chemoattractant is **haptotaxis.** Chemokines are also displayed on cytokine-activated vascular endothelial cells. This process can augment very late antigen-4 (VLA-4) integrin-dependent adhesion of leukocytes, resulting in their firm arrest. As soluble molecules, chemokines control leukocyte motility and localization within extravascular tissues by establishing a chemotactic gradient. The multiplicity and combination of chemokine receptors on cells allows an extensive variety in biological function.

Neutrophils, monocytes, eosinophils, and basophils share some receptors but express other receptors exclusively. Thus specific chemokine combinations can recruit selective cell populations.

Chemokines in Disease
Chemokines are implicated in a variety of acute and chronic diseases. These include disorders with a pronounced inflammatory component, in which case multiple chemokines are expressed in the inflamed tissues. Examples are rheumatoid arthritis, ulcerative colitis, Crohn disease, pulmonary inflammation (chronic bronchitis, asthma), autoimmune diseases (multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus), and vascular diseases, including atherosclerosis.

Reactive Oxygen Species (ROS) Are Signal-Transducing, Bactericidal, and Cytotoxic Molecules

ROS are chemically reactive molecules derived from oxygen. Normally, they are rapidly inactivated, but when generated inappropriately, they can be toxic to cells (see Chapter 1). ROS activate signal-transduction pathways and combine with proteins, lipids, and DNA, a state termed **oxidative stress**, which can lead to loss of cell function and cell death. Leukocyte-derived ROS, released within phagosomes, are bactericidal. ROS important in inflammation include superoxide ($O_2\bullet$, O_2^-), nitric oxide ($NO\bullet$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$) (Fig. 2-16) (see below and Chapter 1).

Superoxide
Molecular oxygen is converted to superoxide anion (O_2^-) by several pathways. (1) Within cells, formation of O_2^- occurs spontaneously near the inner mitochondrial membrane; (2) In vascular endothelial cells, O_2^- is generated by flavoenzymes such as xanthine oxidase, as well as lipoxygenase and cyclooxygenase; (3) In the setting of inflammation, leukocytes, as well as endothelial cells, use a reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to produce O_2^-.

In endothelial cells xanthine oxidase, a purine-metabolizing enzyme, converts xanthine and hypoxanthine to uric acid, thereby generating O_2^-. This pathway is a major intracellular source of O_2^- in neutrophil-mediated cell injury. Proinflammatory mediators, including leukocyte elastase and several cytokines, convert xanthine dehydrogenase to the active xanthine

oxidase. Intracellular O_2^- interacts with nuclear factor NFκB, activating protein-1 (AP-1), and other molecules to activate signal transduction pathways. It is further metabolized to other free radicals, particularly $\bullet OH$, which contribute to inflammation-related cell injury.

The NADPH-oxidase of phagocytic cells, neutrophils, and macrophages is a multicomponent enzyme complex, that generates high concentrations of extracellular and intracellular O_2^-, mainly for bactericidal and cytotoxic functions. This oxidase uses NADH and NADPH as substrates for electron transfer to molecular oxygen. A similar enzyme complex is present in vascular endothelial cells, where it generates significant, albeit lower, concentrations of O_2^-.

Nitric Oxide
Nitric oxide ($NO\bullet$) is synthesized by nitric oxide synthase (NOS), which promotes oxidation of the guanidino nitrogen of L-arginine in the presence of O_2. There are three main NOS isoforms: constitutively expressed neuronal (nNOS) and endothelial (eNOS) forms, and an inducible (iNOS) isoform. Inflammatory cytokines increase expression of iNOS, generating intracellular and extracellular $NO\bullet$. $NO\bullet$ has diverse roles in the physiology and pathophysiology of the vascular system, including:

- $NO\bullet$ generated by eNOS acts as **endothelium-derived relaxing factor** (EDRF), mediating vascular smooth muscle relaxation.

- In physiological concentrations, $NO\bullet$ alone and in balance with O_2^-, is an intracellular messenger.

- $NO\bullet$ prevents platelet adherence and aggregation at sites of vascular injury, reduces leukocyte recruitment, and scavenges oxygen radicals.

- Excessive production of $NO\bullet$, especially in parallel with O_2^-, generates the highly reactive and cytotoxic species, peroxynitrite ($ONOO\bullet$).

Stress Proteins Protect Against Inflammatory Injury
When cells are subjected to stress conditions, many suffer irreversible injury and die, and others are severely damaged. However, mild heat treatment prior to lethal injury provides tolerance to subsequent injury. This phenomenon reflects increased expression of the heat shock family of stress proteins
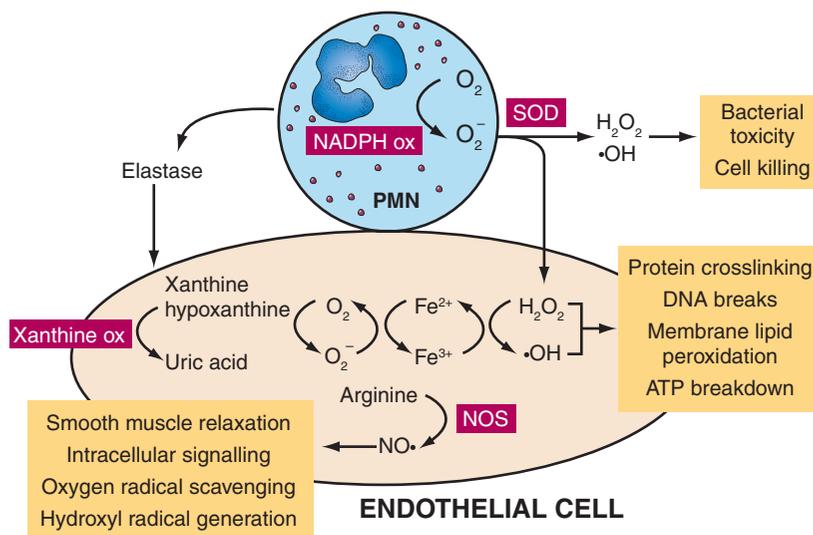
FIGURE 2-16. **Biochemical events in neutrophil-endothelial cell interactions.** When neutrophils are in firm contact with endothelial cells, oxygen radicals and other active molecules generated by both cells interact. Superoxide (O_2^-) generated by the neutrophil NADPH oxidase (NADPH ox) is converted to toxic hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$). Within the endothelial cell, xanthine oxidase oxide (xanthine ox) converts xanthine to uric acid, ultimately generating O_2^- from molecular oxygen. Nitric oxide synthase (NOS) generates nitric oxide ($NO\bullet$) from arginine. Reactive oxygen species contribute to numerous cellular events. ATP = adenosine triphosphate; Fe^{2+} = ferrous iron; Fe^{3+} = ferric iron; PMN = polymorphonuclear neutrophil.

(HSPs). Stress proteins belong to multigene families and are named according to molecular size, for example, Hsp27, Hsp70, and Hsp90. They are upregulated by diverse stresses, including oxidative/ischemic stress and inflammation, and are associated with protection during sepsis and metabolic stress. Protein damage and misfolded proteins are common denominators in injury and disease. Protection from many kinds of nonlethal stresses is mediated by HSPs, which act as molecular chaperones, increasing protein expression by enhancing folding of nascent proteins and preventing misfolding. Potential functions of stress proteins include suppression of proinflammatory cytokines and NADPH oxidase, increased nitric oxide-mediated cytoprotection, and enhanced collagen synthesis.

## Neurokinins Link the Endocrine, Nervous, and Immune Systems

The neurokinin family of peptides includes substance P (SP), neurokinin A (NKA), and neurokinin B (NKB). These peptides are distributed throughout the central and peripheral nervous systems, and represent a link between the endocrine, nervous, and immune systems. A wide range of biological processes is associated with these peptides, including plasma protein extravasation and edema, vasodilation, smooth-muscle contraction and relaxation, salivary secretion, airway contraction, and transmission of nociceptive responses. As early as 1876, Stricker noted an association between sensory afferent nerves and inflammation. *It is now recognized that injury to nerve terminals during inflammation evokes an increase in neurokinins, which in turn influence production of inflammatory mediators, including histamine, NO•, and kinins.* The actions of neurokinins are mediated by activation of at least three classes of receptors—NK1, NK2, and NK3—which are widely distributed throughout the body. The neurokinin system is linked to inflammation in the following settings:

- **Edema formation:** SP, NKA, and NKB induce edema by promoting release of histamine and serotonin from mast cells.
- **Thermal injury:** SP and NKA are produced after thermal injury occurs and mediate early edema.
- **Arthritis:** SP is widely distributed in nerves in joints where it mediates vascular permeability. SP and NKA can modulate the activity of inflammatory and immune cells.
- **Airway inflammation:** SP and NKA have been implicated in bronchoconstriction, mucosal edema, leukocyte adhesion and activation, and increased vascular permeability.

Extracellular Matrix Mediators

The interaction of cells with the extracellular matrix regulates tissue responses to inflammation. The extracellular environment consists of a macromolecular matrix specific to each tissue. During injury, resident inflammatory cells interact with this matrix, using this scaffolding for migration along a chemokine gradient. Collagen, elastic fibers, basement membrane proteins, glycoproteins, and proteoglycans are among the structural macromolecules of the ECM (see Chapter 3). Matricellular proteins are secreted macromolecules that link cells to the ECM or to disrupt cell–ECM interactions. Cytokines and growth factors influence associations among cells, ECM and matricellular proteins (Fig. 2-17). Matricellular proteins include:

- **SPARC (secreted protein acidic and rich in cysteine)** is a multifunctional glycoprotein that organizes ECM compo-
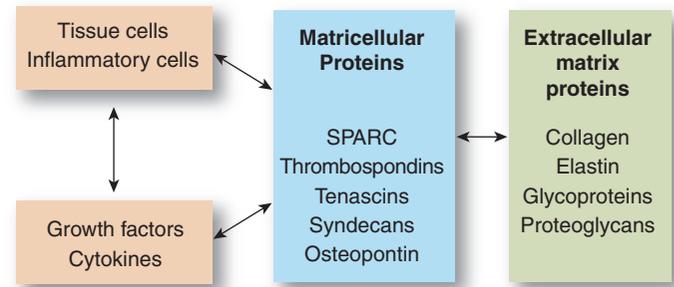
FIGURE 2-17. **Dynamic relationship associates cells, soluble mediators, and matricellular proteins with the extracellular matrix.** SPARC = secreted protein acidic and rich in cysteine.

nents and modulates growth factor activity. It affects cell proliferation, migration, and differentiation, and acts as a counter-adhesive protein, especially on endothelial cells.

- **Thrombospondins** are secreted glycoproteins that modulate cell–matrix interactions, influence platelet aggregation and support neutrophil chemotaxis and adhesion.
- **Tenascins C, X, and R** are counter-adhesive proteins expressed during development, tissue injury, and wound healing.
- **Syndecans** are heparan sulfate proteoglycans implicated in coagulation, growth factor signaling, cell adhesion to the ECM, and tumorigenesis.
- **Osteopontin** is a phosphorylated glycoprotein important in bone mineralization. It also (1) mediates cell–matrix interactions, (2) activates cell signaling (particularly in T cells), (3) is chemotactic for and supports adhesion of leukocytes, and (4) has anti-inflammatory effects via regulation of macrophage function.

Cells of Inflammation

Leukocytes are the major cellular components of the inflammatory response and include neutrophils, T and B lymphocytes, monocytes, macrophages, eosinophils, mast cells, and basophils. Specific functions are associated with each of these cell types, but they overlap and vary as inflammation progresses. In addition, local tissue cells interact with one another and with inflammatory cells, in a continuous response to injury and infection. *Inflammatory cells and resident tissue cells interact during inflammation.* They include neutrophils, endothelial cells, monocyte/macrophages, mast cells, eosinophils, and platelets.

Neutrophils

The polymorphonuclear neutrophil, or (PMN), is the cellular participant in acute inflammation. It has granulated cytoplasm and a nucleus with two to four lobules. PMNs are stored in bone marrow, circulate in the blood, and rapidly accumulate at sites of injury or infection (Fig. 2-18A). They are activated in response to phagocytic stimuli, cytokines, chemotactic mediators, or antigen–antibody complexes that bind specific receptors on their cell membrane. Specifically, neutrophil receptors recognize the Fc portion of IgG and IgM; complement components C5a, C3b, and iC3b; arachidonic acid metabolites (e.g., LTB_4); chemotactic factors (e.g., FMLP, IL-8), and cytokines (e.g., TNF-α). In tissues, PMNs phagocytose invading microbes and dead tissue (see
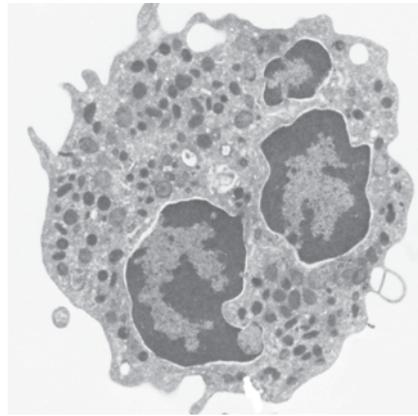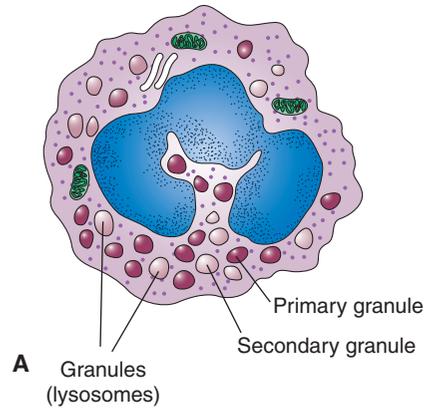
POLYMORPHONUCLEAR LEUKOCYTES

CHARACTERISTICS AND FUNCTIONS
• Central to acute inflammation
• Phagocytosis of microorganisms and tissue debris
• Mediates tissue injury

PRIMARY INFLAMMATORY MEDIATORS
• Reactive oxygen metabolites
• Lysosomal granule contents

Primary granules	**Secondary granules**
Myeloperoxidase	Lysozyme
Lysozyme	Lactoferrin
Defensins	Collagenase
Bactericidal/permeability	Complement activator
increasing protein	Phospholipase A$_2$
Elastase	CD11b/CD18
Cathepsins Protease 3	CD11c/CD18
Glucuronidase	Laminin
Mannosidase	
Phospholipase A$_2$	**Tertiary granules**
	Gelatinase
	Plasminogen activator
	Cathepsins
	Glucuronidase
	Mannosidase

A Granules (lysosomes)
Primary granule
Secondary granule
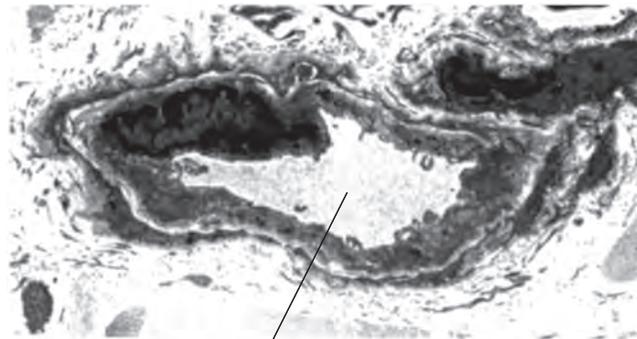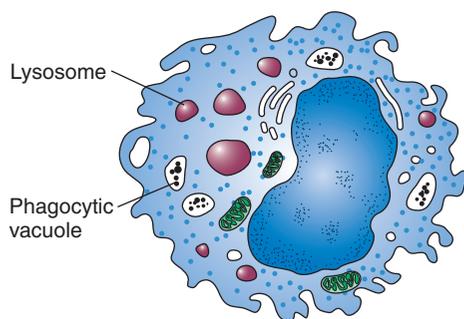
ENDOTHELIAL CELLS

CHARACTERISTICS AND FUNCTIONS
• Maintains vascular integrity
• Regulates platelet aggregation
• Regulates vascular contraction and relaxation
• Mediates leukocyte recruitment in inflammation

PRIMARY INFLAMMATORY MEDIATORS
• von Willebrand factor
• Nitric oxide
• Endothelins
• Prostanoids

B Capillary lumen

MONOCYTE/MACROPHAGE

CHARACTERISTICS AND FUNCTIONS
• Regulates inflammatory response
• Regulates coagulation/fibrinolytic pathway
• Regulates immune response (see Chapt. 4)

PRIMARY INFLAMMATORY MEDIATORS
• cytokines
  -IL-1
  -TNF-α
  -IL-6
  -Chemokines (e.g. IL-8, MCP-1)
• lysosomal enzymes
  -acid hydrolases
  -serine proteases
  -metalloproteases (e.g. collagenase)
• cationic proteins
• prostaglandins/leukotrienes
• plasminogen activator
• procoagulant activity
• oxygen metabolite formation

C Lysosome
Phagocytic vacuole
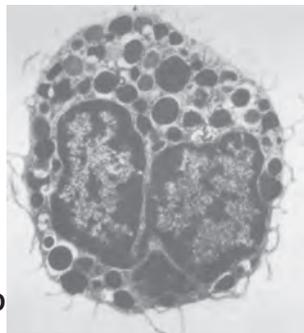
MAST CELL (BASOPHILS)

CHARACTERISTICS AND FUNCTIONS
• Binds IgE molecules
• Contains electron-dense granules

PRIMARY INFLAMMATORY MEDIATORS
• Histamine
• Leukotrienes (LTC, LTD, LTE)
• Platelet activating factor
• Eosinophil chemotactic factors
• Cytokines (e.g., TNF-α IL-4)

D

FIGURE 2-18. Cells of inflammation: morphology, and function. **A.** Neutrophil. **B.** Endothelial cell. **C.** Monocyte/macrophage. **D.** Mast cell. IL = interleukin; MCP-1 = monocyte chemoattractant protein-1 TNF-α = tumor necrosis factor-α.

below). Once they are recruited into tissue, they do not reenter the circulation.

Endothelial Cells

Endothelial cells, a monolayer of cells lining blood vessels, help to separate intra- and extravascular spaces. They produce antiplatelet and antithrombotic agents that maintain blood vessel patency and also vasodilators and vasoconstrictors that regulate vascular tone. Injury to a vessel wall interrupts the endothelial barrier and exposes a local procoagulant signal (Fig. 2-18B).

Endothelial cells are gatekeepers in inflammatory cell recruitment: they can promote or inhibit tissue perfusion and inflammatory cell influx. Inflammatory agents such as bradykinin and histamine, endotoxin, and cytokines induce endothelial cells to reveal adhesion molecules that anchor and activate leukocytes, present major histocompatibility complex (MHC) class I and II molecules, and generate cytokines and important vasoactive and inflammatory mediators. These mediators include:

- **Nitric oxide (NO•):** Originally identified as EDRF, NO• is a low–molecular-weight vasodilator that inhibits platelet aggregation, regulates vascular tone by stimulating smooth muscle relaxation, and reacts with ROS to create highly reactive radical species (see above).

- **Endothelins:** Endothelins-1, -2, and -3 are low–molecular-weight peptides produced by endothelial cells. They are potent vasoconstrictor and pressor agents, which induce prolonged vasoconstriction of vascular smooth muscle.

- **Arachidonic acid-derived contraction factors:** Oxygen radicals generated by the hydroperoxidase activity of cyclooxygenase and prostanoids such as TXA_2 and PGH_2 induce smooth muscle contraction.

- **Arachidonic acid-derived relaxing factors:** The biological opponent of TXA_2, PGI_2 inhibits platelet aggregation and causes vasodilation.

- **Cytokines:** IL-1, IL-6, TNF-α and other inflammatory cytokines are generated by activated endothelial cells.

- **Anticoagulants:** Heparin-like molecules and thrombomodulin inactivate the coagulation cascade (see Chapters 10 and 20).

- **Fibrinolytic factors:** Tissue-type plasminogen activator (t-PA) promotes fibrinolytic activity.

- **Prothrombotic agents:** von Willebrand factor facilitates adhesion of platelets, and tissue factor activates the extrinsic clotting cascade.

Monocyte/Macrophages

Circulating monocytes (Fig. 2-18C) have a single lobed or kidney-shaped nucleus. They are derived from the bone marrow and can exit the circulation to migrate into tissue and become resident macrophages. In response to inflammatory mediators, they accumulate at sites of acute inflammation and take up and process microbes. These cells can also differentiate into dendritic cells, which are highly efficient antigen-presenting cells. Antigens bind to the **major histocompatibility complex** (MHCII) and are presented to lymphocytes, subsequently activating those cells. Monocyte/macrophages produce potent vasoactive mediators, including prostaglandins and leukotrienes, PAF, and inflammatory cytokines. Macrophages are especially important for maintaining chronic inflammation.

Mast Cells and Basophils

Mast cell products play an important role in regulating vascular permeability and bronchial smooth muscle tone, especially in allergic hypersensitivity reactions (see Chapter 4). Granulated mast cells and basophils (Fig. 2-18D) contain cell surface receptors for IgE. Mast cells are found in the connective tissues, and are especially prevalent along lung and gastrointestinal mucosal surfaces, the dermis, and the microvasculature. Basophils circulate in small numbers and can migrate into tissue.

When IgE-sensitized mast cells or basophils are stimulated by antigen; physical agonists, such as cold and trauma; or cationic proteins, inflammatory mediators in their dense cytoplasmic granules are secreted into extracellular tissues. These granules contain acid mucopolysaccharides (including heparin), serine proteases, chemotactic mediators for neutrophils, and eosinophils and histamine, a primary mediator of early increased vascular permeability. Histamine binds specific H_1 receptors in the vascular wall, inducing endothelial cell contraction, gap formation, and edema, an effect that can be inhibited pharmacologically by H_1-receptor antagonists. Stimulation of mast cells and basophils also leads to the release of products of arachidonic acid metabolism, including LTC_4, LTD_4, and LTE_4, and cytokines, such as TNF-α and IL-4.

Eosinophils

Eosinophils circulate in blood and are recruited to tissue similarly to PMNs. They are characteristic of IgE-mediated reactions, such as hypersensitivity, allergic and asthmatic responses (Fig. 2-19A). Eosinophils contain leukotrienes and PAF, as well as acid phosphatase and peroxidase. They express IgA receptors and contain large granules with eosinophil major basic protein, both of which are involved in defense against parasites.
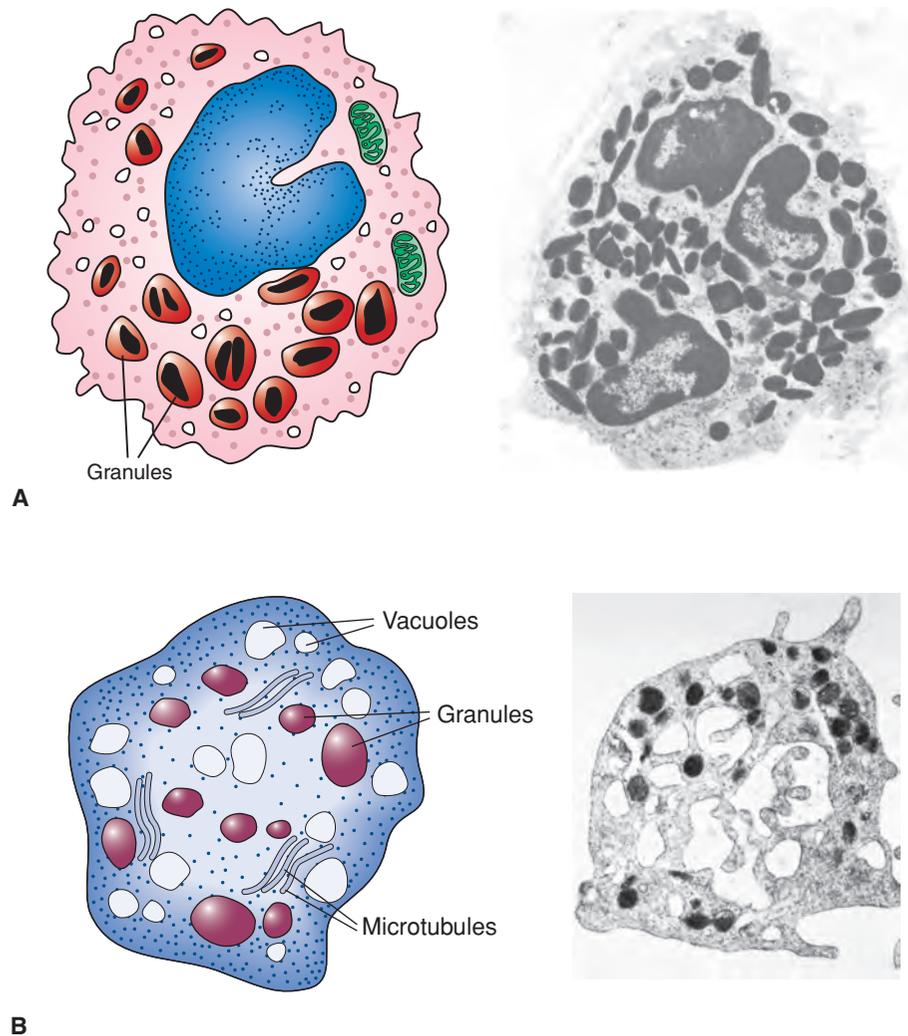
Platelets

Platelets play a primary role in normal homeostasis and in initiating and regulating clot formation (see Chapter 20). They are sources of inflammatory mediators, including potent vasoactive substances and growth factors that modulate mesenchymal cell proliferation (Fig. 2-19B). The platelet is small (2 mm in diameter), lacks a nucleus, and contains three distinct kinds of inclusions: (1) **dense granules**, rich in serotonin, histamine, calcium and adenosine diphosphate (ADP); (2) **α granules**, containing fibrinogen, coagulation proteins, platelet-derived growth factor (PDGF), and other peptides and proteins; and (3) **lysosomes**, which sequester acid hydrolases.

Platelets adhere, aggregate, and degranulate when they contact fibrillar collagen (e.g., after vascular injury that exposes interstitial matrix proteins) or thrombin (after activation of the coagulation system) (Fig. 2-20). Degranulation is associated with release of serotonin (5-hydroxytryptamine), which, like histamine, directly increases vascular permeability. In addition, the arachidonic acid metabolite TXA_2, produced by platelets, plays a key role in the second wave of platelet aggregation and mediates smooth muscle constriction. On activation, platelets, as well as phagocytic cells, secrete cationic proteins that neutralize the negative charges on endothelium and promote increased permeability.

Leukocyte Recruitment in Acute Inflammation

One of the essential features of inflammation is accumulation of leukocytes, particularly PMNs, in affected tissues. Leukocytes adhere to vascular endothelium, becoming activated in the pro-

EOSINOPHILS
CHARACTERISTICS AND FUNCTIONS
• Associated with:
  -Allergic reactions
  -Parasite-associated inflammatory reactions
  -Chronic inflammation
• Modulates mast cell-mediated reactions

PRIMARY INFLAMMATORY MEDIATORS
• Reactive oxygen metabolites
• Lysosomal granule enzymes
  (primary crystalloid granules)
  -Major basic protein
  -Eosinophil cationic protein
  -Eosinophil peroxidase
  -Acid phosphatase
  -β-glucuronidase
  -Arylsulfatase B
  -Histaminase
• Phospholipase D
• Prostaglandins of E series
• Cytokines

PLATELETS

CHARACTERISTICS AND FUNCTIONS
• Thrombosis; promotes clot formation
• Regulates permeability
• Regulates proliferative response of
  mesenchymal cells
PRIMARY INFLAMMATORY MEDIATORS
• Dense granules
  -Serotonin
  -Ca^{2+}
  -ADP
• α-granules
  -Cationic proteins
  -Fibrinogen and coagulation proteins
  -Platelet-derived growth factor (PDGF)
• Lysosomes
  -Acid hydrolases
•Thromboxane A_2

FIGURE 2-19. More cells of inflammation: morphology and function. A. Eosinophil. **B.** Platelet. ADP = adenosine diphosphate.

cess. They then flatten and migrate from the vasculature, through the endothelial cell layer and into surrounding tissue. In the extravascular tissue, PMNs ingest foreign material, microbes and dead tissue (Fig. 2-21).

Leukocyte Adhesion to Endothelium Results from Interaction of Complementary Adhesion Molecules

Leukocyte recruitment to the postcapillary venules begins with interaction of leukocytes with endothelial cell selectins, which are redistributed to endothelial cell surfaces during activation. This interaction, called **tethering**, slows leukocytes in the blood flow (Fig. 2-22). Leukocytes then move along the vascular endothelial cell surface with a saltatory movement, termed **rolling**. PMNs become activated by proximity to the endothelium and by inflammatory mediators, and adhere strongly to intercellular adhesion molecules (ICAMs) on the endothelium (leukocyte **arrest**). As endothelial cells separate, leukocytes **transmigrate** through the vessel wall and, under the influence of chemotactic factors, leukocytes migrate through extravascular tissue to the site of injury.

The events involved in leukocyte recruitment are regulated by (1) inflammatory mediators, which stimulate resident tissue cells, including vascular endothelial cells; (2) expression of adhesion molecules on vascular endothelial cell surfaces, which bind to reciprocal molecules on the surfaces of circulating leukocytes; and (3) chemotactic factors, which attract leukocytes along a chemical gradient to the site of injury.

Adhesion Molecules
Four molecular families of adhesion molecules are involved in leukocyte recruitment: selectins, addressins, integrins, and immunoglobulins (Fig. 2-23).

Selectins
The selectin family includes P-selectin, E-selectin, and L-selectin, expressed on the surface of platelets, endothelial cells, and leukocytes. Selectins share a similar molecular structure, which includes a chain of transmembrane glycoproteins with an extracellular lectin-binding domain. This calcium-dependent or C-type lectin binds to sialylated oligosaccharides, specifically the sialyl-Lewis X
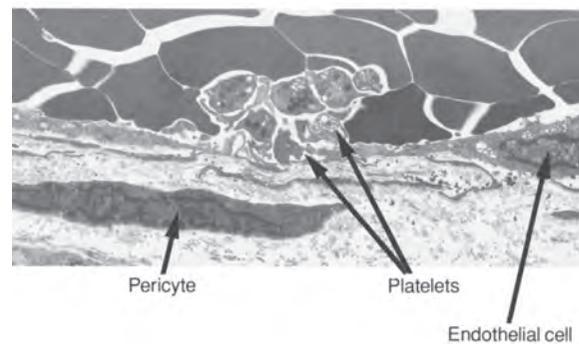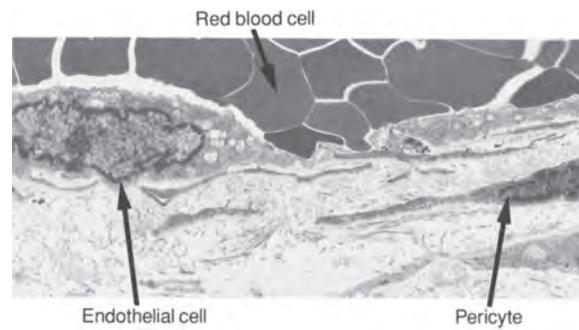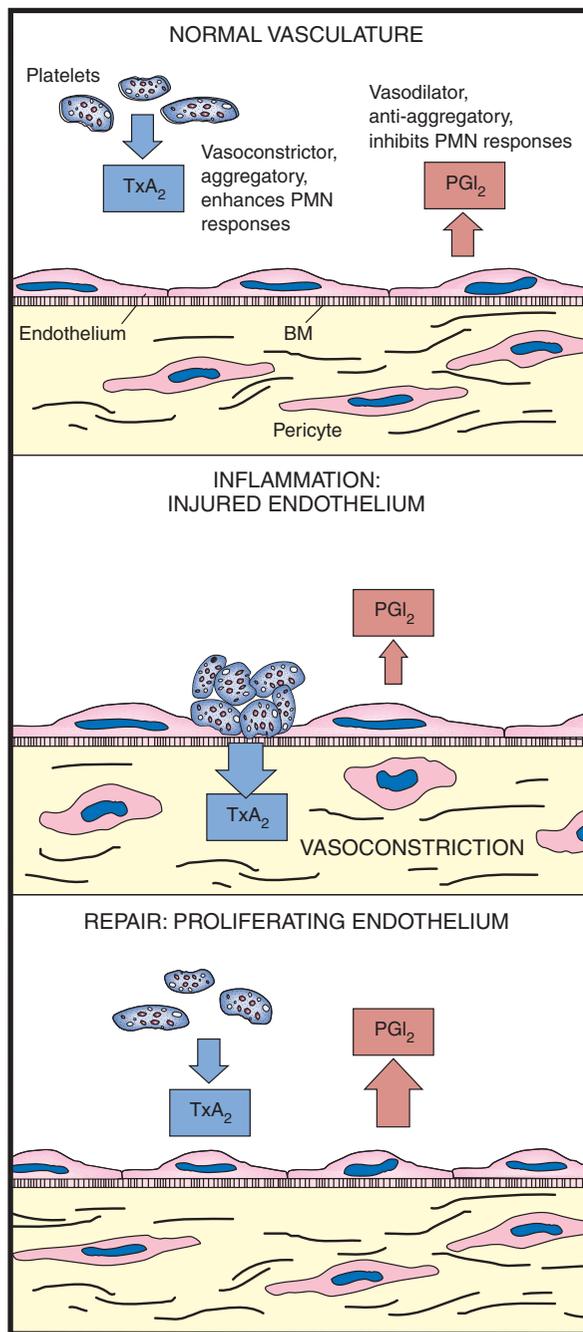
FIGURE 2-20. **Regulation of platelet and endothelial cell interactions by thromboxane A$_2$ *(TXA$_2$)* and pro-staglandin I$_2$ *(PGI$_2$)*.** During inflammation, the normal balance is shifted to vasoconstriction, platelet aggregation, and polymorphonuclear neutrophil (PMN) responses. During repair, the prostaglandin effects predominate. BM = basement membrane.

moiety on addressins, the binding of which allows rapid attachment and rolling of cells.

P-selectin (CD62P, GMP-140, PADGEM) is preformed and stored within Weibel-Palade bodies of endothelial cells and α-granules of platelets. On stimulation with histamine, thrombin, or specific inflammatory cytokines, P-selectin is rapidly transported to the cell surface, where it binds to sialyl-Lewis X on leukocyte surfaces. Preformed P-selectin can be delivered quickly to the cell surface, allowing rapid adhesive interaction between endothelial cells and leukocytes.

E-selectin (CD62E, ELAM-1) is not normally expressed on endothelial cell surfaces but is induced by inflammatory mediators, such as cytokines or bacterial LPS. E-selectin mediates adhesion of neutrophils, monocytes, and certain lymphocytes via binding to Lewis X or Lewis A

L-selectin (CD62L, LAM-1, Leu-8) is expressed on many types of leukocytes. It was originally defined as the "homing receptor" for lymphocytes. It binds lymphocytes to high endothelial venules (HEV) in lymphoid tissue, thereby regulating their trafficking through this tissue. L-selectin binds glycan-bearing cell adhesion molecule-1 (GlyCAM-1), mucosal addressin cell adhesion molecule-1 (MadCAM-1) and CD34.

Addressins

Vascular addressins are mucin-like glycoproteins including GlyCAM-1, P-selectin glycoprotein-1 (PSGL-1), E-Selection ligand (ESL-1), and CD34. They possess sialyl-Lewis X, which binds the lectin domain of selectins. Addressins are expressed at leukocyte and endothelium surfaces. They regulate localization of subpopulations of leukocytes and are involved in lymphocyte activation.
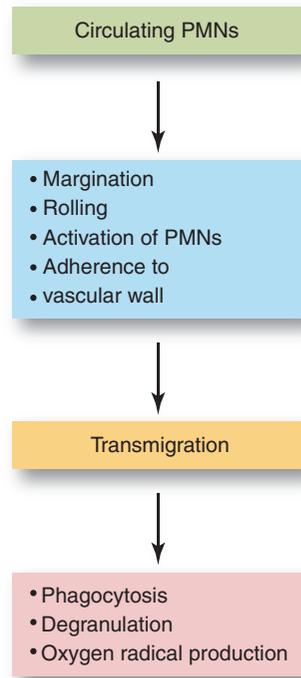
FIGURE 2-21. **Leukocyte recruitment and activation.** PMNs = polymorphonuclear neutrophils.
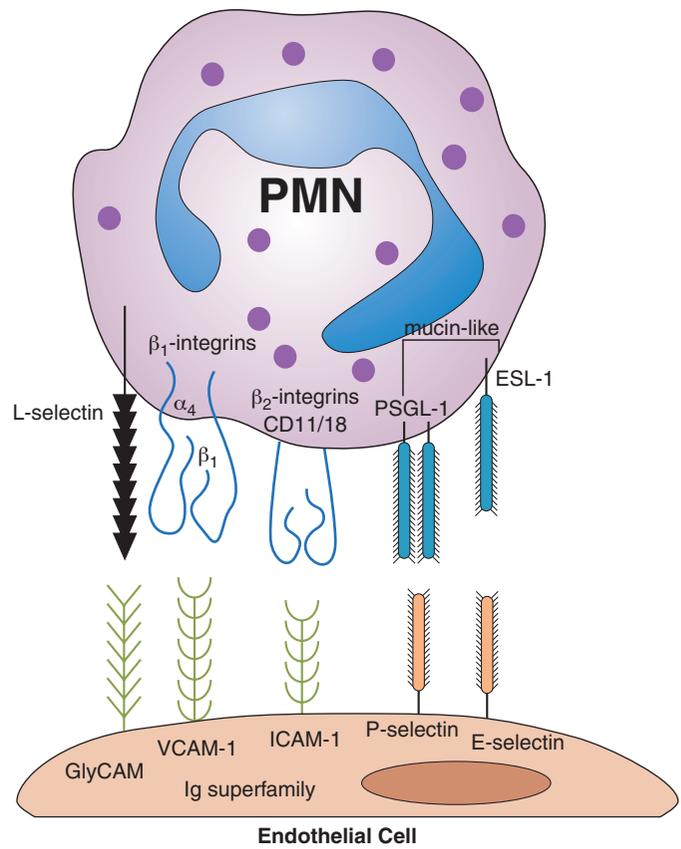
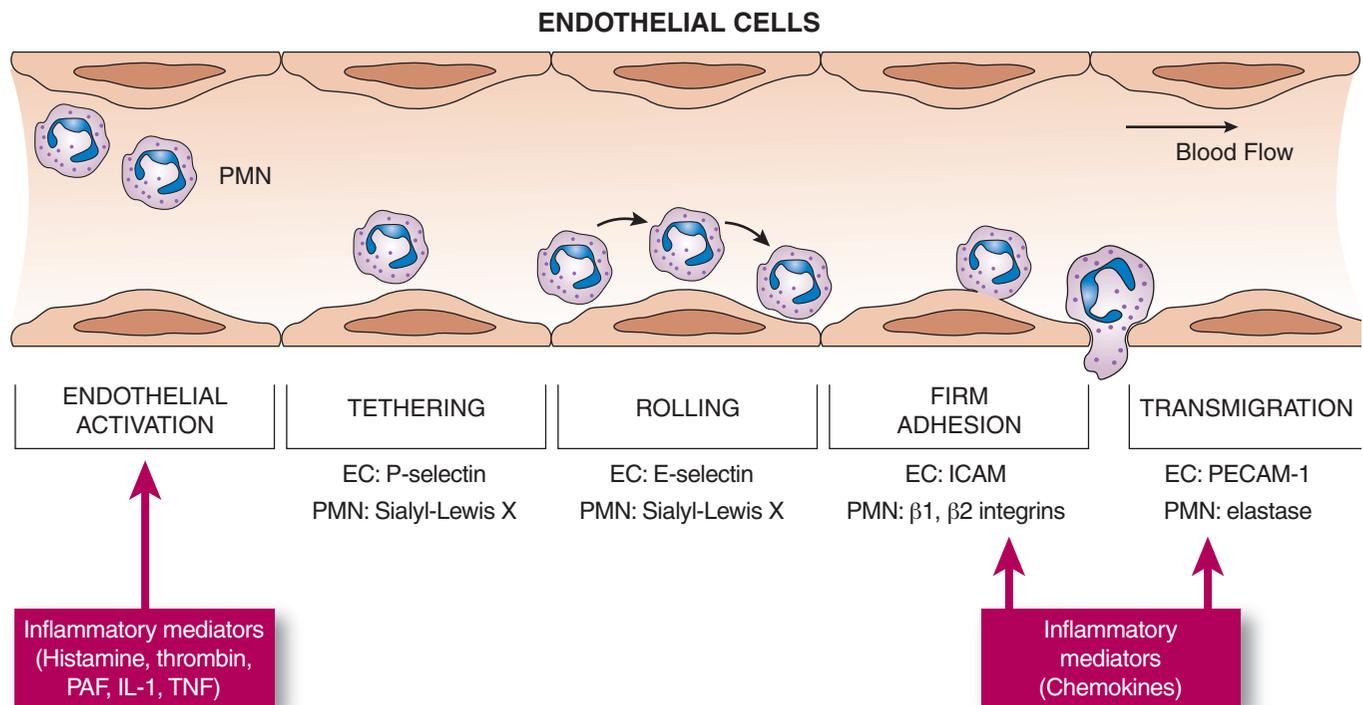FIGURE 2-23. **Leukocyte and endothelial cell adhesion mole-cules.** GlyCAM = glycan-bearing cell adhesion molecule; ICAM-1 = intercellular adhesion molecule-1; VCAM = vascular cell adhesion molecule.

FIGURE 2-22. **Neutrophil adhesion and extravasation.** Inflammatory mediators activate endothelial cells to increase expression of adhesion molecules. Sialyl Lewis X on neutrophil PSGL-1 and ESL-1 binds to P- and E-selectins to facilitate tethering and rolling of neutrophils. Increased integrins on activated neutrophils bind to ICAM-1 on endothelial cells to form a firm attachment. Endothelial cell attachments to one another are released and neutrophils then pass between separated cells to enter the tissue. EC = endothelial cell; ICAM = intercellular adhesion molecule; IL = interleukin; PAF = platelet-activating factor; PMN = polymorphonuclear neutrophil; TNF = tumor necrosis factor.

Integrins

Chemokines, lipid mediators, and proinflammatory molecules activate cells to express the integrin family of adhesion molecules (see Chapter 3). Integrins have transmembrane α and β chains arranged as heterodimers. They participate in cell–cell interactions and cell–ECM binding. β_1, β_2, and β_7 integrins are involved in leukocyte recruitment. Very late activation (VLA) molecules include VLA-4 ($\alpha 4\beta 1$) on leukocytes and lymphocytes that bind vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells. The $\beta 2$ (CD18) integrins form molecules by association with α integrin chains: $\alpha_1\beta_2$ (also called CD11a/CD18 or LFA-1) and $\alpha_m\beta_2$ (also termed CD11b/CD18 or Mac-1) bind ICAM-1 and ICAM-2, respectively. Leukocyte integrins exist in a low affinity state, but are converted to a high affinity state when these cells are activated.

Immunoglobulins

Adhesion molecules of the immunoglobulin superfamily include ICAM-1, ICAM-2, and VCAM-1, all of which interact with integrins on leukocytes to mediate recruitment. They are expressed at the surfaces of cytokine-stimulated endothelial cells and some leukocytes, as well as certain epithelial cells, such as pulmonary alveolar cells.

Recruitment of Leukocytes

Tethering, rolling, and firm adhesion are prerequisites for recruitment of leukocytes from the circulation into tissues. For a rolling cell to adhere, there must first be a selectin-dependent reduction in rolling velocity. The early increase in rolling depends on P-selectin, whereas cytokine-induced E-selectin initiates early adhesion. Integrin family members function cooperatively with selectins to facilitate rolling and subsequent firm adhesion of leukocytes. Leukocyte integrin binding to the Ig superfamily of ligands expressed on vascular endothelium further retard leukocytes, increasing the length of exposure of each leukocyte to endothelium. At the same time, engagement of adhesion molecules activates intracellular signal transduction. As a result, leukocytes and vascular endothelial cells are further activated, with subsequent upregulation of L-selectin and integrin binding. The net result is firm adhesion

Recruitment of specific subsets of leukocytes to areas of inflammation may result from unique patterns or relative densities of adhesion molecules on cell surfaces. For subsets of leukocytes, each cell type can express specific adhesion molecules. Cytokines or chemokines specific to the inflammatory process induce the display of adhesion molecules on vascular endothelium and changes in the affinity of these molecules for their ligands. For example, in allergic or asthmatic inflammation, cytokine induction of VCAM-1 on endothelial cells increases recruitment of the VLA-4–bearing eosinophils in preference to neutrophils, which do not express VLA-4.

Leukocyte recruitment in some tissues may not follow this paradigm. In the liver, leukocytes may not need to roll in the narrow sinusoids before adhering to the endothelium. Leukocyte adherence to arterioles and capillaries also has different requirements, reflecting the different hydrodynamic forces in these vessels.

Chemotactic Molecules Direct Neutrophils to Sites of Injury

Leukocytes must be accurately positioned at sites of inflammatory injury to carry out their biological functions. For specific subsets of leukocytes to arrive in a timely fashion, they must receive very specific directions. *Leukocytes are guided through vascular and extravascular spaces by a complex interaction of attractants, repellants, and adhesion molecules.* **Chemotaxis** is the dynamic and energy-dependent process of directed cell migration. Blood leukocytes are recruited by chemoattractants released by endothelial cells. They then migrate from the endothelium towards the target tissue, down a gradient of one chemoattractant in response to a second more distal chemoattractant gradient.

Neutrophils must integrate the various signals to arrive at the correct site at the correct time to perform their assigned tasks. The most important chemotactic factors for PMNs are C5a, bacterial, and mitochondrial products (particularly low-molecular-weight N-formylated peptides such as FMLP), products of arachidonic acid metabolism, (especially LTB$_4$), products of ECM degradation and, chemokines. The latter represent a key mechanism of leukocyte recruitment because they generate a chemotactic gradient by binding to ECM proteoglycans. As a result, high concentrations of chemokines persist at sites of tissue injury. In turn, specific receptors on migrating leukocytes bind matrix-bound chemokines, moving the cells along the chemotactic gradient to the site of injury.

Chemotactic factors for other cell types, including lymphocytes, basophils, and eosinophils, are also produced at sites of tissue injury and may be secreted by activated endothelial cells, tissue parenchymal cells, or other inflammatory cells. They include PAF, transforming growth factor-β (TGF-β), neutrophilic cationic proteins, and lymphokines. *The cocktail of chemokines presented within a tissue largely determines the type of leukocyte attracted to the site.* Cells arriving at their destination must then be able to stop in the target tissue. Contact guidance, regulated adhesion, or inhibitory signals may determine the final arrest of specific cells in specific tissue locations.

Leukocytes Traverse the Endothelial Cell Barrier to Gain Access to the Tissue

Leukocytes adherent to the vascular endothelium emigrate by **paracellular diapedesis,** i.e., passing between adjacent endothelial cells. Responding to chemokine gradients, neutrophils extend pseudopods and insinuate themselves between the cells and out of the vascular space (Fig. 2-24). Vascular endothelial cells are connected by tight junctions and adherens junctions. CD31 (platelet endothelial cell adhesion molecule [**PECAM-1**]) is expressed on endothelial cell surfaces and binds to itself to keep cells together. These junctions separate under the influence of inflammatory mediators, intracellular signals generated by adhesion molecule engagement and signals from the adherent neutrophils. Neutrophils mobilize elastase to their pseudopod membranes, inducing endothelial cell retraction and separation at the advancing edge of the neutrophil. These cells also induce increases in endothelial cell intracellular calcium, to which they respond by pulling apart.

Neutrophils also migrate through endothelial cells, by **transcellular diapedesis**. Instead of inducing endothelial cell retraction PMNs may squeeze through small circular pores in endothelial cell cytoplasm. In tissues that contain fenestrated microvessels, such as gastrointestinal mucosa and secretory glands, PMNs may traverse thin regions of endothelium, called **fenestrae**, without damaging endothelial cells. In nonfenestrated microvessels, PMNs may cross the endothelium using endothelial cell caveolae or pinocytotic vesicles, which form small, membrane-bound passageways across the cell.
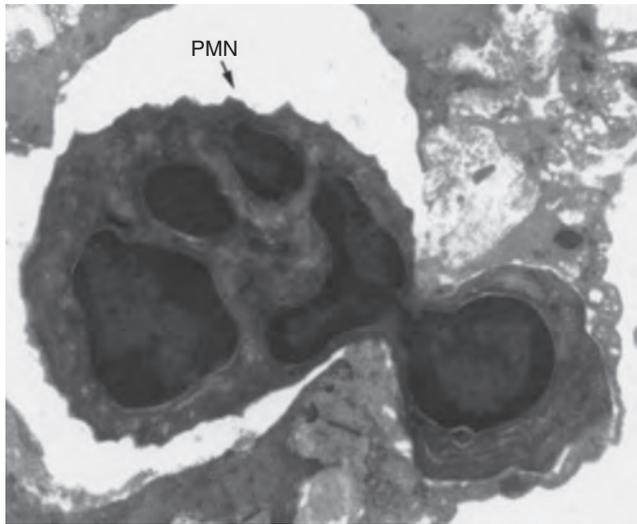
FIGURE 2-24. **Transmission electron micrograph demonstrates neutrophil transmigration.** A neutrophil exits the vascular space by diapedesis across the vascular endothelium. PMN = polymorphonuclear neutrophil.

Leukocyte Functions in Acute Inflammation

Leukocytes Phagocytose Microorganisms and Tissue Debris

Many inflammatory cells—including monocytes, tissue macrophages, dendritic cells, and neutrophils—recognize, internalize, and digest foreign material, microorganisms, or cellular debris by a process termed **phagocytosis**. This term was first used over a century ago by Elie Metchnikoff and is now defined as ingestion by eukaryotic cells of large (usually > 0.5 μm) insoluble particles and microorganisms. The effector cells are **phagocytes**. The complex process involves a sequence of transmembrane and intracellular signaling events.

1. **Recognition:** Phagocytosis is initiated by recognition of particles by specific receptors on the surface of phagocytic cells (Fig. 2-25). Phagocytosis of most biological agents is enhanced by, if not dependent on, their coating (**opsonization**) with plasma components (**opsonins**), particularly immunoglobulins or C3b. Phagocytic cells possess specific opsonic receptors, including those for immunoglobulin Fcγ and complement components. Many pathogens, however, have evolved mechanisms to evade phagocytosis by leukocytes. Polysaccharide capsules, protein A, protein M, or peptidoglycans around bacteria can prevent complement deposition or antigen recognition and receptor binding.

2. **Signaling:** Clumping of opsonins at bacterial surfaces causes phagocyte plasma membrane Fcγ receptors to cluster. Subsequent phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs), located in the cytosolic domain or γ subunit of the receptor, trigger intracellular signaling events. Tyrosine kinases that associate with the Fcγ receptor are required for signaling during phagocytosis (Fig. 2-26).

3. **Internalization:** For Fcγ receptor or CR3, actin assembly occurs directly under the phagocytosed target. Polymerized actin filaments push the plasma membrane forward. The

PHAGOSOME FORMATION
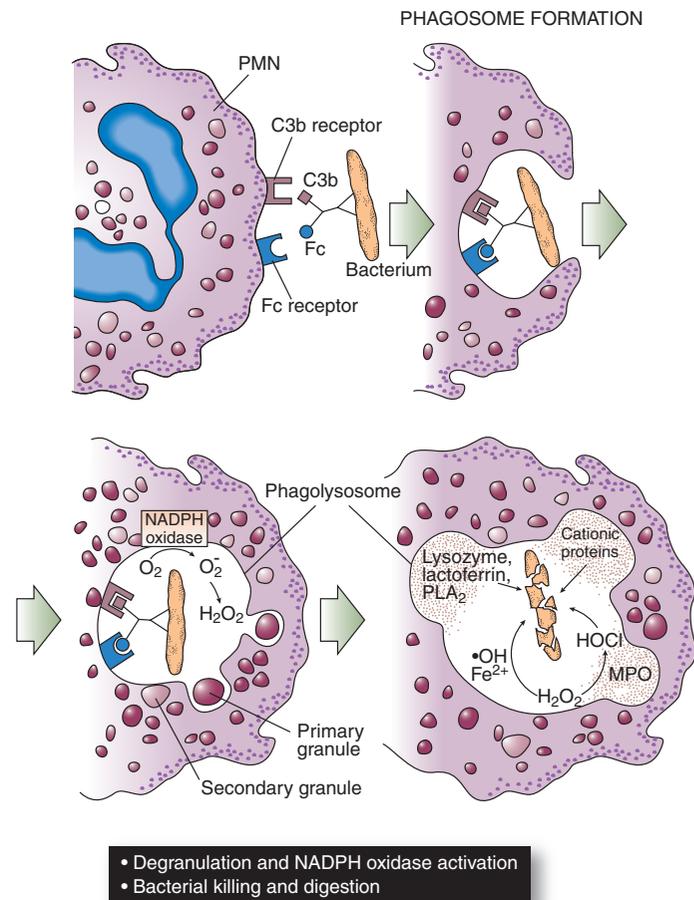
- Degranulation and NADPH oxidase activation
- Bacterial killing and digestion

FIGURE 2-25. **Mechanisms of neutrophil bacterial phagocytosis and cell killing.** Opsonins such as C3b coat the surface of microbes allowing recognition by the neutrophil C3b receptor. Receptor clustering triggers intracellular signalling and actin assembly within the neutrophil. Pseudopods form around the microbe to enclose it within a phagosome. Lysosomal granules fuse with the phagosome to form a phagolysosome into which the lysosomal enzymes and oxygen radicals are released to kill and degrade the microbe. Fe^{2+} = ferrous iron; HOCl = hypochlorous acid; MPO = myeloperoxidase; PLA_2 = phospholipase A_2; PMN = polymorphonuclear neutrophil.

plasma membrane remodels to increase surface area and to form pseudopods surrounding the foreign material. The resulting phagocytic cup engulfs the foreign agent. The membrane then "zippers" around the opsonized particle to enclose it in a cytoplasmic vacuole called a **phagosome** (see Figs. 2-25 and 2-26).

4. **Digestion:** The phagosome that contains the foreign material fuses with cytoplasmic lysosomes to form a **phagolysosome**, into which lysosomal enzymes are released. The acid pH within the phagolysosome activates these hydrolytic enzymes, which then degrade the phagocytosed material. Some microorganisms have evolved mechanisms for evading killing by neutrophils by preventing lysosomal degranulation or inhibiting neutrophil enzymes.

Neutrophil Enzymes Are Required for Antimicrobial Defense and Débridement

Although PMNs are critical for degrading microbes and cell debris, they also contribute to tissue injury (Fig. 2-27). PMN release
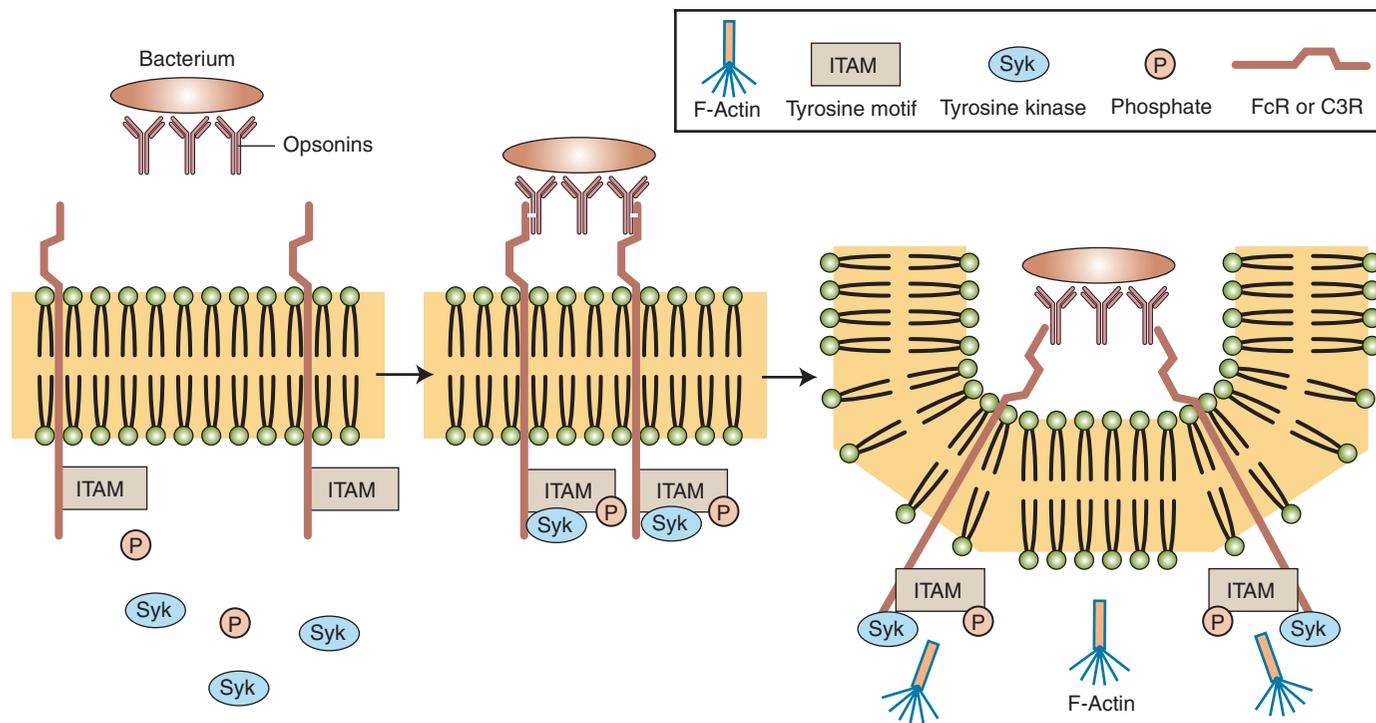
FIGURE 2-26. Intracellular signaling during leukocyte phagocytosis. Opsonins coating the surface of microbes or foreign material are recognized by the neutrophil C3b receptor. Receptor clustering triggers phosphorylation of ITAMs on the receptor and tyrosine kinases initiate intracellular signalling. Polymerized actin filament, aggregate beneath the plasma membrane to form a pseudopod to enclose the foreign agent.

of their granule contents at sites of injury is a double-edged sword. On the one hand, débridement of damaged tissue by proteolytic breakdown is beneficial. On the other hand, degradative enzymes can damage endothelial and epithelial cells, as well degrade connective tissue.
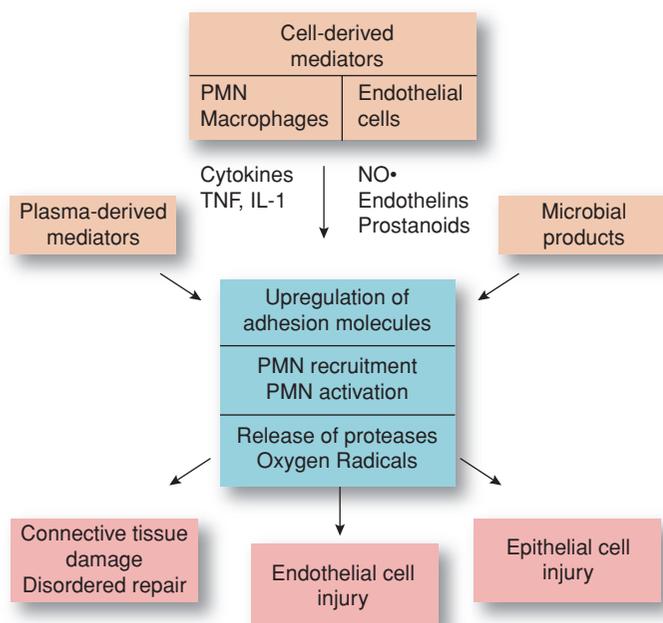
FIGURE 2-27. Leukocyte-mediated inflammatory injury. IL = interleukin; LPS = lipopolysaccharide; NO•= nitric oxide; PMN = polymorphonuclear neutrophil; TNF = tumor necrosis factor.

Neutrophil Granules

The armamentarium of enzymes required for degradation of microbes and tissue is generated and contained within PMN cytoplasmic granules. Neutrophil primary, secondary, and tertiary granules are differentiated morphologically and biochemically: each granule has a unique spectrum of enzymes (see Fig. 2-18A).

- **Primary granules (azurophilic granules):** Antimicrobial and proteinase activity of these granules can directly activate other inflammatory cells. Potent acid hydrolases and neutral serine proteases digest a number of macromolecules. Lysozyme and PLA_2 degrade bacterial cell walls and biological membranes and are important in killing bacteria. Myeloperoxidase, a key enzyme in the metabolism of hydrogen peroxide, generates toxic oxygen radicals.

- **Secondary granules (specific granules):** These contain PLA_2, lysozyme, and proteins that initiate killing of specific cells. In addition, their contents include the cationic protein, lactoferrin, a vitamin B_{12}-binding protein and a matrix metalloproteinase (collagenase) specific for type IV collagen.

- **Tertiary granules (small storage granules, C granules):** These granules are released at the leading front of neutrophils during chemotaxis. They are the source of enzymes that promote migration of cells through basement membranes and tissues including proteinases cathepsin, gelatinase, and urokinase-type plasminogen activator (u-PA).

Proteinases

Proteolytic enzymes (proteinases) cleave peptide bonds in polypeptides; they are stored in cytoplasmic granules and secretory vesicles of neutrophils. As these cells emerge from the circulation, they release proteinases that enable them to penetrate

the ECM and migrate to sites of injury and there degrade matrix, cell debris, and pathogens. Neutrophils, however, are not the only source of proteinases. These enzymes are also made by most inflammatory cells, including monocytes, eosinophils, basophils, mast cells, and lymphocytes, as well as tissue cells, including vascular endothelial cells.

Proteinases are classified by their catalytic activity into four groups: serine proteinases and metalloproteinases are neutral enzymes that function in extracellular spaces; cysteine proteinases and aspartic proteinases are acidic and act in the acidic milieu of lysosomes (Table 2-3). These enzymes target a variety of intracellular and extracellular proteins, including (1) inflammatory products; (2) debris from damaged cells, microbial proteins, and matrix proteins; (3) microorganisms; (4) plasma proteins, including complement components, clotting factors, immunoglobulins, and cytokines; (5) matrix macromolecules (e.g., collagen, elastin, fibronectin, and laminin); and (6) lymphocytes and platelets.

Serine Proteinases

Serine proteinases degrade extracellular proteins, cell debris and bacteria. Human leukocyte elastase (HLE) is primarily responsible for fibronectin degradation. Cathepsin G (CG) converts angiotensin I to angiotensin II, thereby mediating smooth muscle contraction and vascular permeability. Proteinase 3 (PR3) has antigenic properties related to Wegener granulomatosis. u-PA dissolves fibrin clots to generate plasmin at wound sites, degrades ECM proteins, and activates procollagenases to create a path for leukocyte migration. Although serine proteinases are most important in digesting ECM molecules, they also modify cytokine activity: they solubilize membrane-bound cytokines and receptors by cleaving active cytokines from inactive precursors. They also detach cytokine receptors from cell surfaces, thus regulating cytokine bioactivity.

Metalloproteinases

There are at least 25 members identified (see Chapter 3). Matrix metalloproteinases (**MMPs**, matrixins) degrade all ECM compo-nents, including basement membranes. They are subclassified according to substrate specificity into interstitial collagenases, gelatinases, stromelysins, metalloelastases, and matrilysin. Proteins with disintegrin and metalloproteinase domains (ADAMs) regulate neutrophil infiltration by targeting the distintegrins. These molecules are polypeptides that disrupt integrin-mediated binding of cells to each other and to ECM.

Cysteine Proteinases and Aspartic Proteinases

These acid proteinases function primarily within lysosomes of leukocytes to degrade intracellular proteins.

Proteinase Inhibitors

The proteolytic environment is regulated by a battery of inhibitors. During wound healing, these antiproteases protect against damage by limiting protease activity. ECM remodeling occurs in the context of a balance between enzymes and inhibitors. In chronic wounds, continuous influx of neutrophils, with their proteases and ROS, may overwhelm and inactivate these inhibitors, allowing continuation of proteolysis (see Chapter 3). Known proteinase inhibitors include:

- α_2-**Macroglobulin:** Nonspecific inhibitor of all classes of proteinases, primarily found in plasma
- **Serpins:** The major inhibitors of serine proteinases
- α_1-**Antiproteases** (α_1-**antitrypsin,** α_1-**antichymotrypsin**): Inhibit human leukocyte elastase and cathepsin G
- **Secretory leukocyte proteinase inhibitor (SLPI), Elafin:** Inhibit proteinase 3
- **Plasminogen activator inhibitors (PAIs):** Inhibit u-PA
- **Tissue inhibitors of metalloproteinases (TIMP-1,-2,-3, -4):** Specific for matrix metalloproteinases in tissue

Inflammatory Cells Have Oxidative and Nonoxidative Bactericidal Activity

The bactericidal activity of PMNs and macrophages is mediated in part by production of ROS and in part by oxygen-independent mechanisms.

Bacterial Killing by Oxygen Species

Phagocytosis is accompanied by metabolic reactions in inflammatory cells that lead to production of several oxygen metabolites (see Chapter 1). These products are more reactive than oxygen itself and contribute to the killing of ingested bacteria (see Fig. 2-25).

- **Superoxide anion** (O_2^-): Phagocytosis activates a NADPH oxidase in PMN cell membranes. NADPH oxidase is a multicomponent electron transport complex that reduces molecular oxygen to O_2^-. Activation of this enzyme is enhanced by prior exposure of cells to a chemotactic stimulus or LPS. NADPH oxidase activation increases oxygen consumption and stimulates the hexose monophosphate shunt. Together, these cell responses are referred to as the **respiratory burst**.
- **Hydrogen peroxide** (H_2O_2): O_2^- is rapidly converted to H_2O_2 by superoxide dismutase at the cell surface and in phagolysosomes. H_2O_2 is stable and serves as a substrate for generating additional reactive oxidants.
- **Hypochlorous acid** (HOCl): Myeloperoxidase (MPO), a neutrophil product with a very strong cationic charge, is secreted from granules during exocytosis and, in the presence

TABLE 2–3	
Proteinases in Inflammation	
Enzyme Class	**Examples**
Neutral Proteinases	
Serine proteinases	Human leukocyte elastase Cathepsin G Proteinase 3 Urokinase-type plasminogen activator
Metalloproteinase	Collagenases (MMP-1, MMP-8, MMP-13) Gelatinases (MMP-7, MMP-9) Stromelysins (MMP-3, MMP-10, MMP-11) Matrilysin (MMP-7) Metalloelastase (MMP-12) ADAMs-7,-9,-15,-17
Acidic Proteinases	
Cysteine proteinases Aspartic proteinases	Cathepsins, S, L, B, H Cathepsin D

MMP: matrix metalloproteinase; ADAM: proteins with *A Di*sintegrin and *A M*etalloproteinase domain

of a halide, usually chlorine, catalyzes conversion of H_2O_2 to HOCl. This powerful oxidant is a major bactericidal agent produced by phagocytic cells. HOCl also participates in activating neutrophil-derived collagenase and gelatinase, both of which are secreted as latent enzymes. HOCl also inactivates α_1-antitrypsin.

- **Hydroxyl radical** (•OH): Reduction of H_2O_2 occurs via the Haber-Weiss reaction to form the highly reactive •OH. This reaction occurs slowly at physiological pH, but in the presence of ferrous iron (Fe^{2+}) the Fenton reaction rapidly converts H_2O_2 to •OH, a radical with potent bactericidal activity. Further reduction of •OH leads to formation of H_2O (see Chapter 1).

- **Nitric oxide** (NO•): Phagocytic cells and vascular endothelial cells produce NO• and its derivatives, which have diverse effects, both physiological and nonphysiological. NO• and other oxygen radical species interact with one another to balance their cytotoxic and cytoprotective effects. NO• can react with oxygen radicals to form toxic molecules such as peroxynitrite and *S*-nitrosothiols, or it can scavenge O_2^-, thereby reducing the amount of toxic radicals.

Monocytes, macrophages and eosinophils also produce oxygen radicals, depending on their state of activation and the stimulus to which they are exposed. Production of ROS by these cells contributes to their bactericidal and fungicidal activity, and their ability to kill certain parasites. The importance of oxygen-dependent mechanisms in bacterial killing is exemplified in **chronic granulomatous disease** of childhood. In this hereditary deficiency of NADPH oxidase, failure to produce O_2^- and H_2O_2 during phagocytosis makes these persons susceptible to recurrent infections, especially with gram-positive cocci. Patients with a related genetic deficiency in MPO cannot produce HOCl, and show increased susceptibility to infections with the fungal pathogen *Candida* (Table 2-4).

TABLE 2–4

Congenital Diseases of Defective Phagocytic Cell Function Characterized by Recurrent Bacterial Infections

Disease	Defect
Leukocyte adhesion deficiency (LAD)	LAD-1 defective β_2-integrin expression or function (CD11/CD18) LAD-2 (defective fucosylation, selectin binding)
Hyper-IgE-recurrent infection, (Job) syndrome	Poor chemotaxis
Chediak-Higashi syndrome	Defective lysosomal granules, poor chemotaxis
Neutrophil-specific granule deficiency	Absent neutrophil granules
Chronic granulomatous disease	Deficient NADPH oxidase, with absent H_2O_2 production
Myeloperoxidase deficiency	Deficient HOCl production

H_2O_2 = hydrogen peroxide; HOCl = hypochlorous acid; Ig = immunoglobulin.

Nonoxidative Bacterial Killing

Phagocytes, particularly PMNs and monocytes/macrophages, have substantial antimicrobial activity which is oxygen-independent. This activity mainly involves preformed bactericidal proteins in cytoplasmic granules. These include lysosomal acid hydrolases and specialized noncatalytic proteins unique to inflammatory cells.

- **Lysosomal hydrolases:** Neutrophil primary and secondary granules, and lysosomes of mononuclear phagocytes contain hydrolases, including sulfatases and phosphatases, and other enzymes capable of digesting polysaccharides and DNA.

- **Bactericidal/permeability-increasing protein (BPI):** This cationic protein in PMN primary granules can kill many gram-negative bacteria but is not toxic to gram-positive bacteria or to eukaryotic cells. BPI inserts into the outer membrane of bacterial envelopes and increases its permeability. Activation of certain phospholipases and enzymes then degrades bacterial peptidylglycans.

- **Defensins:** Primary granules of PMNs and lysosomes of some mononuclear phagocytes contain this family of cationic proteins, which kill a extensive variety of gram-positive and gram-negative bacteria, fungi, and some enveloped viruses. Some of these polypeptides also can also kill host cells. Defensins are chemotactic for phagocytic leukocytes, immature dendritic cells, and lymphocytes, so they help to mobilize and amplify antimicrobial immunity.

- **Lactoferrin:** Lactoferrin is an iron-binding glycoprotein found in the secondary granules of neutrophils, and in most body secretory fluids. Its iron-chelating capacity allows it to compete with bacteria for iron. It may also facilitate oxidative killing of bacteria by enhancing •OH formation.

- **Lysozyme:** This bactericidal enzyme is found in many tissues and body fluids, in primary and secondary granules of neutrophils and in lysosomes of mononuclear phagocytes. Peptidoglycans of gram-positive bacterial cell walls are exquisitely sensitive to degradation by lysozyme; gram-negative bacteria are usually resistant to it.

- **Bactericidal proteins of eosinophils:** Eosinophils contain several granule-bound cationic proteins, the most important of which are major basic protein (MBP) and eosinophilic cationic protein. MBP accounts for about half of the total protein of the eosinophil granule. Both proteins are ineffective against bacteria but are potent cytotoxic agents for many parasites.

Defects in Leukocyte Function

The importance of protection afforded by acute inflammatory cells is emphasized by the frequency and severity of infections when PMNs are greatly decreased or defective. *The most common such deficit is iatrogenic neutropenia resulting from cancer chemotherapy.* Functional impairment of phagocytes may occur at any step in the sequence: adherence, emigration, chemotaxis, or phagocytosis. These disorders may be acquired or congenital. Acquired diseases, such as leukemia, diabetes mellitus, malnutrition, viral infections, and sepsis, are often accompanied by defects in inflammatory cell function. Table 2-4 shows representative examples of congenital diseases linked to defective phagocytic function.

REGULATION OF INFLAMMATION

Plasma- and cell-derived proinflammatory mediators described above amplify tissue responses and represent a positive feedback loop, with progressive amplification of the response and subsequent tissue injury. Left unchecked, this intense inflammatory injury leads to organ failure. Complement factors, proinflammatory cytokines, and in some cases, immune complexes, activate signal transduction pathways that control gene expression of proinflammatory mediators, including TNF-α, IL-1, chemokines, and adhesion molecules. Secreted cytokines then propagate the response by activating other cell types using these and similar pathways.

While the response of cells and tissues is primarily proinflammatory, endogenous mediators control the extent of inflammatory injury by negative feedback inhibition of proinflammatory gene transcription, thereby preventing uncontrolled inflammation. Particularly important endogenous regulators of inflammation include:

- **Cytokines:** IL-6, IL-10, IL-11, IL-12, and IL-13 limit inflammation by reducing production of the powerful proinflammatory cytokine, TNF-α. In some instances, this effect occurs by preventing degradation of IκB (the NFκB inhibitor), thereby inhibiting cell activation and further release of inflammatory mediators.

- **Protease inhibitors:** SLPI and TIMP-2 are particularly important in reducing the responses of a variety of cell types, including macrophages and endothelial cells, and in decreasing connective tissue damage.

- **Lipoxins:** Lipoxins and aspirin-triggered lipoxins are anti-inflammatory lipid mediators that inhibit leukotriene biosynthesis.

- **Glucocorticoids:** Stimulation of the hypothalamic-pituitary-adrenal axis leads to release of immunosuppressive glucocorticoids. These have transcriptional and post-transcriptional suppressive effects on inflammatory response genes.

- **Kininases:** Kininases in plasma and blood degrade the potent proinflammatory mediator bradykinin.

- **Phosphatases:** One of the most common mechanisms used in signal transduction to regulate inflammatory cell signaling is rapid and reversible protein phosphorylation. Phosphatases and associated regulatory proteins provide a balancing, dephosphorylating system.

Common Intracellular Pathways Are Associated with Inflammatory Cell Activation

The process by which diverse stimuli lead to the functional responses of inflammatory cells is referred to as **stimulus–response coupling**. Stimuli can include microbial products and the many plasma- or cell-derived inflammatory mediators described in this chapter. Although intracellular signaling pathways are complex and vary with cell type and stimulus, some common intracellular pathways are associated with inflammatory cell activation, including G protein, TNF receptor (TNFR), and JAK-STAT pathways (Figs. 2-28, 2-29, 2-30, respectively)

G PROTEIN PATHWAYS: Many chemokines, hormones, neurotransmitters, and other inflammatory mediators signal via guanine nucleotide-binding (G) proteins. G proteins vary in their
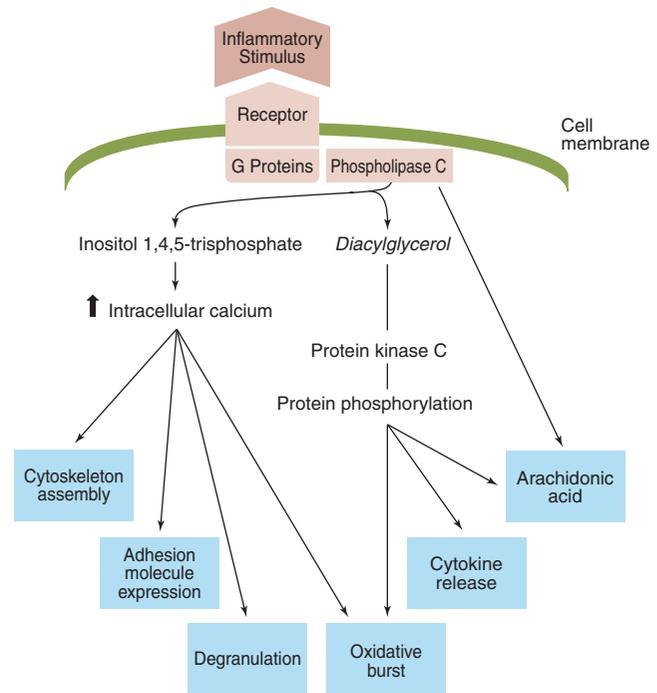

FIGURE 2-28. G-protein–mediated intracellular signal transduction pathway common to many inflammatory stimuli.

intracellular connections, but common activities include (see Fig. 2-28):

- **Ligand–receptor binding:** Binding of a stimulatory factor to a specific cell membrane receptor creates a ligand–receptor complex. Exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) activates the G protein, which dissociates into subunits that, in turn, activate phospholipase C and phosphatidylinositol-3-kinase (PI-3-kinase).

- **Phospholipid metabolism of cell membranes:** Phospholipase C hydrolyzes a phosphoinositide in the plasma mem-
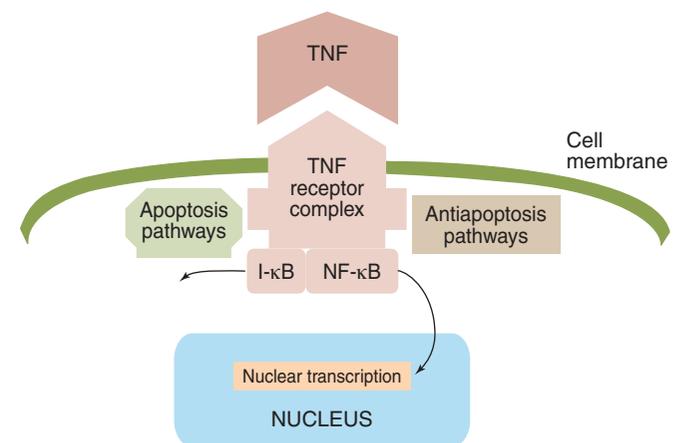
FIGURE 2-29. TNF receptor-mediated intracellular signal transduction pathway. TNF = tumor necrosis factor
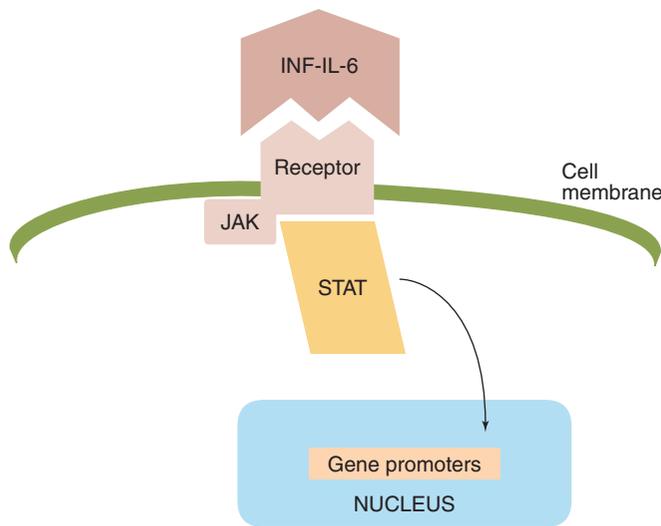
FIGURE 2-30. **JAK-STAT–mediated intracellular transduction pathway.** INF = interferon; IL – interleukin.

brane (phosphatidylinositol bisphosphate [PIP_2]), to generate diacylglycerol and inositol trisphosphate (IP_3).

- **Elevated cytosolic free calcium:** IP_3 induces release of stored intracellular calcium. In conjunction with an influx of calcium ions from the extracellular environment, IP_3 increases cytosolic free calcium, a key event in inflammatory cell activation.

- **Protein phosphorylation and dephosphorylation:** Specific tyrosine kinases bind the ligand–receptor complex and initiate a series of protein phosphorylations.

- **Protein kinase C activation:** Protein kinase C and other protein kinases activate several intracellular signaling pathways, often leading to gene transcription.

TNFR PATHWAYS: TNF is central to the development of inflammation and its symptoms. It also induces tumor cell apoptosis and regulates immune functions (see Fig. 2-29). TNF and related proteins interact with two cell surface receptors to form a multiprotein-signaling complex at the cell membrane. This complex can trigger (1) apoptosis-related enzymes, **caspases** (see Chapter 1) ; (2) inhibitors of apoptosis; or (3) activation of a nuclear transcription factor, **NFκB**. NFκB activation is regulated by association with and disassociation from IκB. IκB binding to NFκB prevents the latter from translocating to the nucleus, where it can function as a transcriptional activator. This latter pathway is critical to regulation of TNF-mediated events during inflammation.

JAK-STAT PATHWAYS: This pathway provides a direct signaling route from extracellular polypeptides (e.g., growth factors) or cytokines (e.g., interferons or interleukins) through cell receptors to gene promoters in the nucleus. Ligand–receptor interactions generate transcription complexes composed of JAK-STAT (Janus kinase-signal transducer and activator of transcription proteins). STAT proteins translocate to the nucleus, where they interact with gene promoters (see Fig. 2-30).

The quantity and quality of inflammatory mediators affects the responses to invading microbes, allergens, or foreign proteins. Regulation occurs at each step of the signal transduction pathways described above, including receptor expression, transcription, and post-transcriptional events.

TRANSCRIPTIONAL CONTROL: Gene promoters for induction of chemokines, adhesion molecules, COX-2, NOS, and collagenase may bind NFκB, AP-1, C/EBP (CAAT/enhancer-binding protein), and ETS transcriptional activators.

POST-TRANSCRIPTIONAL REGULATION: Alterations in mRNA stability have been implicated in suppression of translation of mediators such as TNF-α.

The outcome of these signaling mechanisms and gene transcription is induction or enhancement of specific functional responses, including phagocytosis, degranulation, cell and platelet aggregation, oxidant production, adhesion molecule expression, and cytokine production. Genetics, as well as sex and age of a patient, determines the variable response to injury and in particular, the progression to chronic inflammatory disease. An understanding of inflammatory cell stimulation and regulation provides the basis for new strategies for therapeutic manipulation of both beneficial and harmful inflammatory processes.

Outcomes of Acute Inflammation

As a result of regulatory components and the short life span of neutrophils, acute inflammatory reactions are usually self-limiting and resolve. Resolution involves removal of dead cells, clearance of acute response cells, and reestablishment of the stroma. However inflammatory responses can lead to other outcomes:

- **Resolution:** Under ideal conditions the source of tissue injury is eliminated, inflammation resolves, and normal tissue architecture and physiological function are restored. The progression of inflammation depends on the balance of cell recruitment, cell division, cell emigration, and cell death. For tissue to return to normal, this process must be reversed: the stimulus to injury removed, proinflammatory signals turned off, acute inflammatory cell influx ended, tissue fluid balance restored, cell and tissue debris removed, normal vascular function restored, epithelial barriers repaired, and the ECM regenerated. As signals for acute inflammation wane, PMN apoptosis limits the immune response and resolution begins.

- **Scar:** If a tissue is irreversibly injured, normal architecture is often replaced by a scar, despite elimination of the initial pathological insult (see Chapter 3).

- **Abscess:** If the area of acute inflammation is walled off by inflammatory cells and fibrosis, PMN products destroy the tissue, forming an abscess.

- **Lymphadenitis:** Localized acute inflammation and chronic inflammation may cause secondary inflammation of lymphatic channels (**lymphangitis**) and lymph nodes (**lymphadenitis**). The inflamed lymphatic channels in the skin appear as red streaks, and the lymph nodes are enlarged and painful. Microscopically, the lymph nodes show lymphoid follicle hyperplasias and proliferation of mononuclear phagocytes in the sinuses (sinus histiocytosis).

- **Persistent inflammation:** Failure to eliminate a pathological insult or inability to trigger resolution results in a persistent inflammatory reaction. This may be evident as a prolonged acute response, with continued influx of neutrophils and tissue destruction, or more commonly as chronic inflammation.

Chronic Inflammation

When acute inflammation does not resolve or becomes disordered, chronic inflammation occurs. Inflammatory cells persist, stroma responds by becoming hyperplastic, and tissue destruction and scarring lead to organ dysfunction. This process may be

localized, but more commonly progresses to disabling diseases such as chronic lung disease, rheumatoid arthritis, asthma, ulcerative colitis, granulomatous diseases, autoimmune diseases, and chronic dermatitis. Acute and chronic inflammation are ends of a dynamic continuum with overlapping morphological features: (1) inflammation with continued recruitment of chronic inflammatory cells is followed by (2) tissue injury due to prolongation of the inflammatory response, and (3) an often disordered attempt to restore tissue integrity. The events leading to an amplified inflammatory response resemble those of acute inflammation in a number of aspects:

- **Specific triggers,** microbial products or injury, initiate the response.
- **Chemical mediators** direct recruitment, activation, and interaction of inflammatory cells. Activation of coagulation and complement cascades generate small peptides that function to prolong the inflammatory response. Cytokines, specifically IL-6 and RANTES, regulate a switch in chemokines, such that mononuclear cells are directed to the site. Other cytokines (e.g., IFN-γ) then promote macrophage proliferation and activation.
- **Inflammatory cells** are recruited from the blood. Interactions between lymphocytes, macrophages, dendritic cells, and fibroblasts generate antigen-specific responses.
- **Stromal cell activation and extracellular matrix** remodeling occur, both of which affect the cellular immune response. Varying degrees of fibrosis may result, depending on the extent of tissue injury and persistence of the pathological stimulus and inflammatory response.

Chronic inflammation is not synonymous with chronic infection, but if the inflammatory response cannot eliminate an injurious agent, infection may persist. Chronic inflammation does not necessarily require infection: it may follow an acute inflammatory or immune response to a foreign antigen. Signals that result in an extended response include:

- **Bacteria, viruses, and parasites:** These agents can provide signals to support persistence of the inflammatory response, which in this case may be directed towards isolating the invader from the host.
- **Trauma:** Extensive tissue damage releases mediators capable of inducing an extended inflammatory response.
- **Cancer:** Chronic inflammatory cells, especially macrophages and T lymphocytes, may be the morphological expression of an immune response to malignant cells. Chemotherapy may suppress normal inflammatory responses, increasing susceptibility to infection.
- **Immune factors:** Many autoimmune diseases, including rheumatoid arthritis, chronic thyroiditis, and primary biliary cirrhosis, are characterized by chronic inflammatory responses in affected tissues. This may be associated with activation of antibody-dependent and cell-mediated immune mechanisms (see Chapter 4). Such autoimmune responses may account for injury in affected organs.

Cells Involved in Chronic Inflammation

The cellular components of chronic inflammatory responses are recruited from the circulation (macrophages, lymphocytes, plasma cells, dendritic cells, and eosinophils) and affected tissues (fibroblasts, vascular endothelial cells).

Monocyte/Macrophages

Activated macrophages and their cytokines are central to initiating inflammation and prolonging responses that lead to chronic inflammation. (see Fig. 2-18C). Macrophages produce inflammatory and immunological mediators, and regulate reactions leading to chronic inflammation. They also regulate lymphocyte responses to antigens and secrete other mediators that modulate fibroblast and endothelial cell proliferation and activities

The **mononuclear phagocyte system** includes promonocytes and their precursors in the bone marrow, blood monocytes, and different types of histiocytes and macrophages, particularly Kupffer cells. Under the influence of chemotactic stimuli, IFN-γ (interferon-γ) and bacterial endotoxins, resident tissue macrophages are activated and proliferate, while circulating monocytes are recruited and differentiate into tissue macrophages (Fig. 2-31).

Within different tissues, resident macrophages differ in their armamentarium of enzymes and can respond to local inflammatory signals. Blood monocyte granules contain serine proteinases like those found in neutrophils. Circulating monocytes synthesize additional enzymes, particularly MMPs. When monocytes enter tissue and further differentiate into macrophages, they acquire the ability to generate additional MMPs and cysteine proteinases but lose the ability to produce serine proteinases. The activity of these enzymes is central to the tissue destruction in chronic inflammation. In emphysema, for example, resident macrophages generate proteinases, particularly MMPs with elastolytic activity, which destroy alveolar walls and recruit blood monocytes into the lung. Other macrophage products include oxygen metabolites, chemotactic factors, cytokines, and growth factors

Lymphocytes

Naïve lymphocytes home to secondary lymphoid organs, where they encounter antigen-presenting cells and become antigen-specific lymphocytes. Plasma cells and T cells leaving secondary lymphoid organs circulate in the vascular system and are recruited into peripheral tissues.
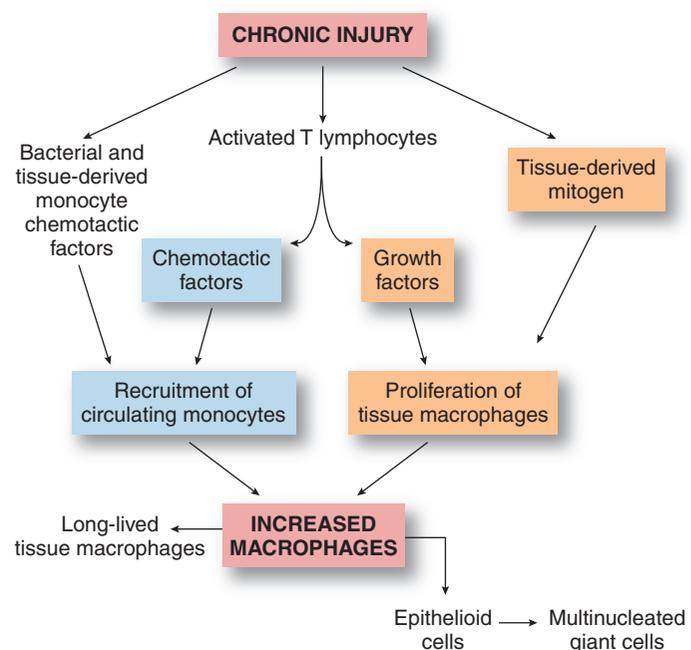

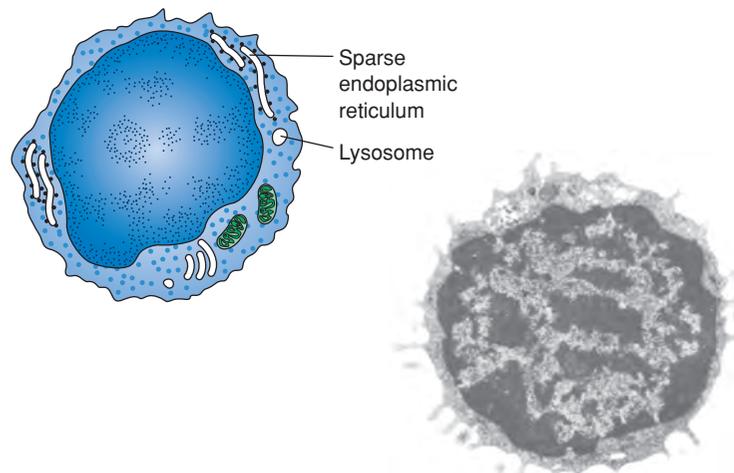FIGURE 2-31. **Accumulation of macrophages in chronic inflammation.**

CHARACTERISTICS AND FUNCTIONS
- Associated with chronic inflammation
- Key cells in humoral and cell-mediated immune responses
- Cytokine production
- Multiple subtypes:

B cell ⟶ Plasma cell ⟶ Antibody production

Effector cells ⟶
- Delayed hypersensitivity
- Mixed lymphocyte reactivity
- Cytotoxic "killer" cells (K-cells)

T cell ⟶

Regulatory cells ⟶
- Helper T cells
- Suppressor T cells

Cytotoxic natural killer (NK) cell
Null cell

FIGURE 2-32. **Lymphocyte: Morphology and function.**

T-cells regulate macrophage activation and recruitment by secreting specific mediators (lymphokines), modulate antibody production and cell-mediated cytotoxicity, and maintain immunologic memory (Fig. 2-32). NK cells, as well as other lymphocyte subtypes, help defend against viral and bacterial infections.

Plasma Cells

Plasma cells are rich in rough endoplasmic reticulum and are the primary source of antibodies (Fig. 2-33). The production of antibody to specific antigens at sites of chronic inflammation is important in antigen neutralization, clearance of foreign antigens, and particles and antibody-dependent cell-mediated cytotoxicity (see Chapter 4).

Dendritic Cells

Dendritic cells are professional antigen-presenting cells that trigger immune responses to antigens (see Chapter 4). They phagocytose antigens and migrate to lymph nodes, where they present those antigens. Recognition of antigen and other costimulatory molecules by T cells results in recruitment of specific cell subsets to the inflammatory process. During chronic inflammation, dendritic cells are present in inflamed tissues, where they help prolong responses.

Acute Inflammatory Cells

Neutrophils are characteristically involved in acute inflammation, but may also be present during chronic inflammation, if there is ongo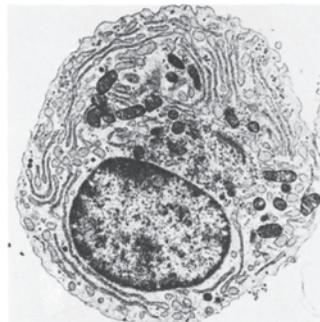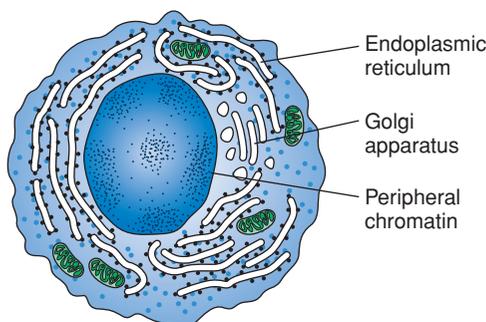ing infection and tissue damage. Eosinophils are particularly prominent in allergic-type reactions and parasitic infestations.

Fibroblasts

Fibroblasts are long-lived, ubiquitous cells whose chief function is to produce components of the ECM (Fig. 2-34). They are derived from mesoderm or neural crest and can differentiate into other connective tissue cells, including chondrocytes, adipocytes, osteocytes, and smooth muscle cells. Fibroblasts are the construction workers of the tissue, rebuilding the scaffolding of ECM upon which tissue is reestablished.

Fibroblasts not only respond to immune signals that induce their proliferation and activation but are also active players in the immune response. They interact with inflammatory cells, particularly lymphocytes, via surface molecules and receptors on both cells. For example, when CD40 on fibroblasts binds its ligand on lymphocytes, both cells are activated. Activated fibroblasts produce cytokines, chemokines, and prostanoids, creating a tissue microenvironment that further regulates the behavior of inflammatory cells in the damaged tissue. (Fibroblast function in wound healing is discussed more fully in Chapter 3.

Injury and Repair in Chronic Inflammation

Chronic inflammation is mediated by both immunological and nonimmunological mechanisms, and is frequently observed in conjunction with reparative responses, namely, granulation tissue and fibrosis.

Endoplasmic reticulum

Golgi apparatus

Peripheral chromatin

CHARACTERISTICS AND FUNCTIONS
- Associated with:
  - antibody synthesis and secretion
  - chronic inflammation
- Derived from B lymphocytes

FIGURE 2-33. **Plasma cell: Morphology and function.**

Endoplasmic Reticuliun

Collagen fibrils

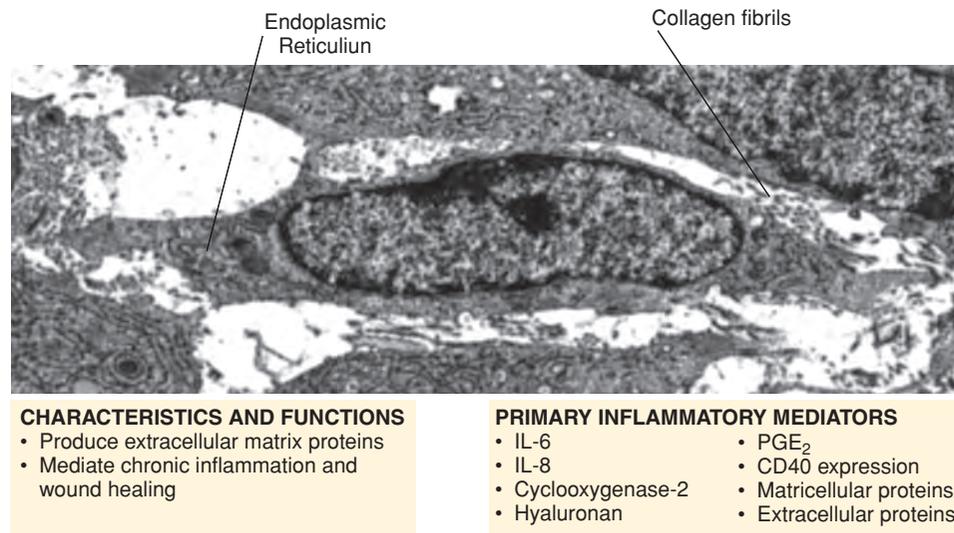| CHARACTERISTICS AND FUNCTIONS	PRIMARY INFLAMMATORY MEDIATORS	
• Produce extracellular matrix proteins	• IL-6	• PGE$_2$
• Mediate chronic inflammation and	• IL-8	• CD40 expression
wound healing	• Cyclooxygenase-2	• Matricellular proteins
	• Hyaluronan	• Extracellular proteins

FIGURE 2-34. **Fibroblast: Morphology and function.** IL = interleukin.

An Extended Inflammatory Response may Lead to Persistent Injury

The primary role of neutrophils in inflammation is host defense and débridement of damaged tissue. The neutrophil response, however, is a double-edged sword: neutrophil products protect the host by participating in antimicrobial defense and débridement of damaged tissue; however, these same products may prolong tissue damage and promote chronic inflammation if they are not appropriately regulated. Neutrophil enzymes are beneficial when they are digesting phagocytosed organisms intracellularly, but can be destructive if they are released extracellularly. Thus, when neutrophils accumulate connective tissue may be digested by their enzymes.

Persistent tissue injury produced by inflammatory cells is important in the pathogenesis of several diseases, for instance, pulmonary emphysema, rheumatoid arthritis, certain immune complex diseases, gout, and adult respiratory distress syndrome. Phagocytic cell adherence, escape of reactive oxygen metabolites and release of lysosomal enzymes together enhance cytotoxicity and tissue degradation. Proteinase activity is significantly elevated in chronic wounds, creating a proteolytic environment that prevents healing.

Altered Repair Mechanisms Prevent Resolution

Repair processes initiated as part of the inflammation can restore normal architecture and function. Early reparative efforts mimic wound healing. However, when inflammation is prolonged or exaggerated, repair is incompletely effective and altered tissue architecture and tissue dysfunction result. These might include:

• Ongoing proliferation of epithelial cells can result in **metaplasia**. For example, goblet cell metaplasia characterizes the airways of smokers and asthmatics.

• Fibroblast proliferation and activation results in increased ECM. Because ECM components such as collagen now occupy space normally devoted to tissue cells, organ function is altered (see Chapter. 3).

• The ECM may be abnormal. Degradation and production of the matrix change the normal mix of extracellular proteins.
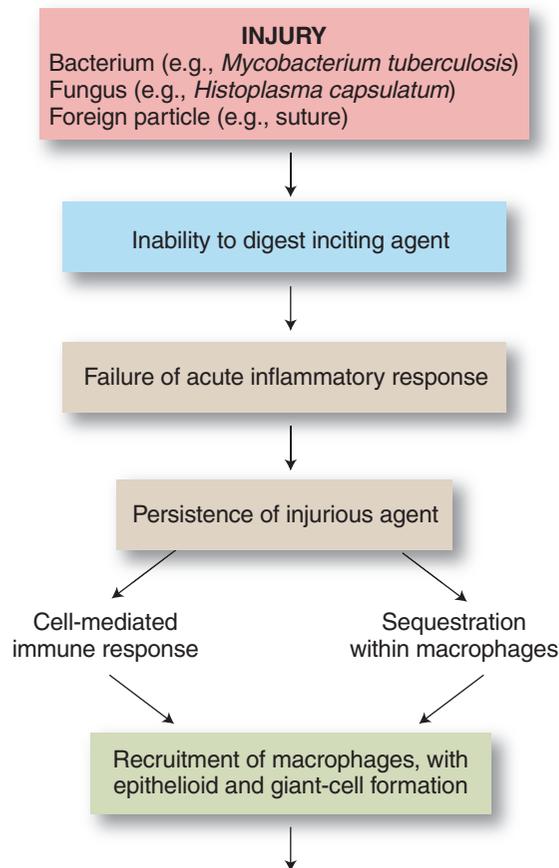
For example, elastin degradation plays an important role in development of emphysema.

• Altered ECM (e.g., fibronectin) can be a chemoattractant for inflammatory cells and present an altered scaffolding to cells.

Granulomatous Inflammation

Neutrophils ordinarily remove agents that incite an acute inflammatory response. However, there are circumstances in which reactive neutrophils cannot digest the substances that provoke acute inflammation. Such a situation is potentially dangerous, because it can lead to a vicious circle of (1) phagocytosis, (2) failure of digestion, (3) death of the neutrophil, (4) release of the undigested provoking agent, and (5) re-phagocytosis by a newly recruited neutrophil (Fig. 2-35). *Granuloma formation is a protective response to chronic infection (fungal infections, tuberculosis, leprosy, schistosomiasis) or the presence of foreign material (e.g., suture or talc), preventing dissemination and restricting inflammation, thereby protecting the host tissues.* Some autoimmune diseases are also associated with granulomas, diseases such as rheumatoid arthritis and Crohn's disease In some cases such as sarcoidosis, no inciting agent has yet been identified.
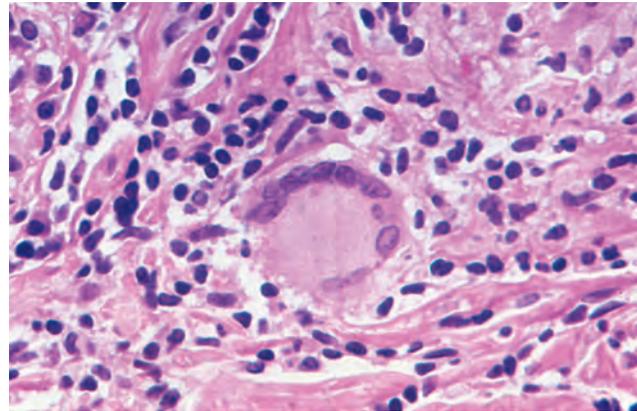
The principal cells involved in granulomatous inflammation are macrophages and lymphocytes (Fig. 2-36). Macrophages are mobile cells that continuously migrate through the extravascular connective tissues. After amassing substances that they cannot digest, macrophages lose their motility, accumulate at the site of injury, and undergo transform themselves into nodular collections of pale, epithelioid cells: the **granuloma**. Multinucleated giant cells are formed by the cytoplasmic fusion of macrophages. When the nuclei of such giant cells are arranged around the periphery of the cell in a horseshoe pattern, the cell is called a **Langhans giant cell** (Fig. 2-37). Frequently, a foreign agent (e.g., silica or a *Histoplasma* spore) or other indigestible material is identified within the cytoplasm of a multinucleated giant cell, in which case the term **foreign body giant cell** is used (Fig. 2-38). Foreign body giant cells tend to have more centrally situated nuclei. Granulomas are further classified histopathologically by the presence or absence of necrosis. Certain infectious
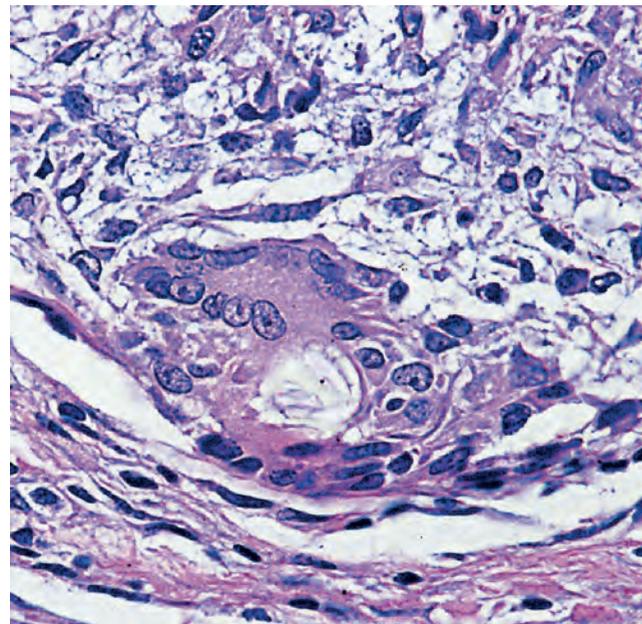
INJURY
Bacterium (e.g., *Mycobacterium tuberculosis*)
Fungus (e.g., *Histoplasma capsulatum*)
Foreign particle (e.g., suture)

↓

Inability to digest inciting agent

↓

Failure of acute inflammatory response

↓

Persistence of injurious agent

↙ ↘

Cell-mediated
immune response   Sequestration
within macrophages

↘ ↙

Recruitment of macrophages, with
epithelioid and giant-cell formation

↓

FIGURE 2-35. **Mechanism of granuloma formation.**

FIGURE 2-37. **A Langhans giant cell shows nuclei arranged on the periphery of an abundant cytoplasm.**

FIGURE 2-38. **A foreign body giant cell has numerous nuclei randomly arranged in the cytoplasm.**

agents such as *Mycobacterium tuberculosis* characteristically produce necrotizing granulomas, the centers of which are filled with an amorphous mixture of debris and dead microorganisms and cells. Other diseases such as sarcoidosis are characterized by granulomas that lack necrosis.
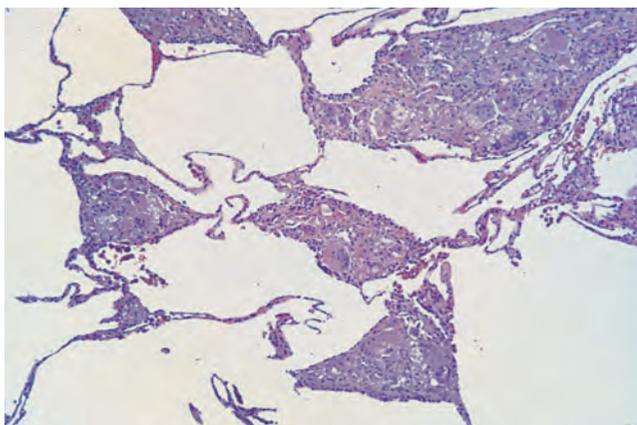
Immune granulomas, formed during delayed type hypersensitivity responses contain activated T cells and macrophages, which initiate granuloma formation. CD4+ T cells then recruit and organize cells at the site using CXCL chemokines to develop
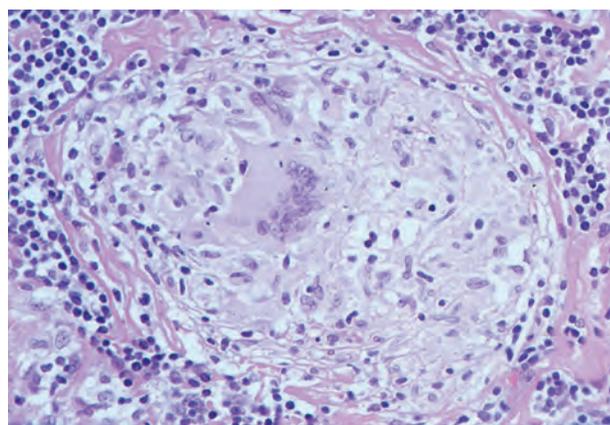

FIGURE 2-36. **Granulomatous inflammation. A.** Section of lung from a patient with sarcoidosis reveals numerous discrete granulomas. **B.** A higher-power photomicrograph of a single granuloma in a lymph node from the same patient depicts a multinucleated giant cell amid numerous pale epithelioid cells. A thin rim of fibrosis separates the granuloma from the lymphoid cells of the node.

Th1 type granulomas and CCL chemokines to develop Th2 type granulomas. Several T cell cytokines stimulate macrophage function (e.g., IFN-γ), whereas others inhibit macrophage activation (e.g., IL-4, IL-10). Thus, lymphocytes are vital for regulating development and resolution of inflammatory responses.

The fate of a granulomatous reaction depends on the toxicity and immunogenicity of the inciting agent. Cell-mediated immune responses to an inciting agent may modify a granulomatous reaction by recruiting and activating more macrophages and lymphocytes. Finally, under the influence of T cell cytokines such as IL-13 and TGFβ, the granuloma burns out and becomes a fibrotic nodule.

Chronic Inflammation and Malignancy

Several chronic infectious diseases are associated with development of malignancies. For example, schistosomiasis of the urinary bladder leads to cancer of that organ. Inflammation that is not specifically linked to infection may also be a risk factor for cancer. Patients with reflux esophagitis or ulcerative colitis are at higher risk for cancer in those organs. The environment created by chronic inflammation promotes malignant transformation by a number of mechanisms (see also Chapter 5):

- **Increased cell proliferation:** Chronically stimulated cell division increases the likelihood of transforming mutations in the proliferating cells
- **Oxygen and NO• metabolites:** Inflammatory metabolites, such as nitrosamines, may cause genomic damage.
- **Chronic immune activation:** Chronic antigen exposure induces an altered cytokine profile, suppressing cell-mediated immune responses and creating an environment permissive for malignant growth.
- **Angiogenesis:** Growth of new vessels is associated with inflammation and wound healing and is required for maintenance of neoplastic lesions.
- **Inhibition of apoptosis:** Chronic inflammation suppresses apoptosis. Increased cell division and decreased apoptosis facilitate survival and expansion of mutated cell populations.

Systemic Manifestations of Inflammation

An effective inflammatory response will: (1) confine the area of injury, (2) clear the inciting pathological agent and damaged tissue, and (3) restore tissue function. However, under certain conditions, local injury may result in prominent systemic effects that can themselves be debilitating. These effects often result when a pathogen enters the bloodstream, a condition known as **sepsis**. Cytokines—including IL-1α, IL-1β, TNF-α, IL-6—and interferons, often acting synergistically, are directly or indirectly responsible for both local and systemic effects of inflammation. The symptoms associated with inflammation, including fever, myalgia, arthralgia, anorexia, and somnolence, are attributable to these cytokines. The most prominent systemic manifestations of inflammation, termed the **systemic inflammatory response syndrome** (**SIRS**), are activation of the hypothalamic-pituitary-adrenal axis, leukocytosis, or the acute phase response, fever, and shock.

Hypothalamic-Pituitary-Adrenal Axis

Many of the systemic effects of inflammation are mediated via the hypothalamic-pituitary-adrenal axis, a key component in the response to chronic inflammation and chronic immune disease.

Inflammation results in release of anti-inflammatory glucocorticoids from the adrenal cortex. Loss of adrenal function can increase the severity of inflammation.

Leukocytosis

Leukocytosis, defined as an increase in circulating leukocytes, commonly accompanies acute inflammation. Immature PMNs, ("band" forms) may also be seen in the peripheral blood (see Chapter 20). It is most commonly associated with bacterial infections and tissue injury. Leukocytosis is caused by release of specific mediators from macrophages and perhaps other cells. These mediators accelerate release of PMNs, even immature PMNs, from the bone marrow. Subsequently, macrophages and T lymphocytes are stimulated to produce a group of proteins (called "colony-stimulating factors") that induce proliferation of bone marrow hematopoietic precursor cells. On occasion, circulating levels of leukocytes and their precursors may reach very high levels, a situation referred to as a **leukemoid reaction**, which is sometimes difficult to differentiate from leukemia.

In contrast to bacterial infections, viral infections (including infectious mononucleosis) are characterized by **lymphocytosis**, an absolute increase in the number of circulating lymphocytes. Parasitic infestations and certain allergic reactions cause eosinophilia (i.e., increased eosinophils in the peripheral blood).

Leukopenia

Leukopenia is an absolute decrease in circulating white cells. It is occasionally encountered under conditions of chronic inflammation, especially in patients who are malnourished or who suffer from a chronic debilitating disease such as disseminated cancer. Leukopenia may also be caused by typhoid fever and certain viral and rickettsial infections.

Acute Phase Response

The acute phase response is a regulated physiological reaction that occurs in inflammatory conditions. It is characterized clinically by fever, leukocytosis, decreased appetite, and altered sleep patterns, and chemically by changes in plasma levels of acute phase proteins. These proteins (Table 2-5) are synthesized primarily by the liver and released in large numbers into the circulation in response to an acute inflammatory challenge. Changes in plasma levels of acute phase proteins are mediated primarily by IL-1, IL-6 and TNF-α. Increased plasma levels of some acute

TABLE 2–5	
Acute Phase Proteins	
Protein	**Function**
Mannose binding protein	Opsonization/ complement activation
C-reactive protein	Opsonization
α_1-Antitrypsin	Serine protease inhibitor
Haptoglobin	Binds hemoglobin
Ceruloplasmin	Antioxidant, binds copper
Fibrinogen	Coagulation
Serum amyloid A protein	Apolipoprotein
α_2-Macroglobulin	Antiprotease
Cysteine protease inhibitor	Antiprotease

phase proteins lead to accelerated **erythrocyte sedimentation rate** (ESR), which is a qualitative index used clinically to monitor the activity of many inflammatory diseases.

Fever

Fever is a clinical hallmark of inflammation. Release of **pyrogens** (molecules that cause fever) by bacteria, viruses or injured cells may directly affect the hypothalamic thermoregulation. More importantly, they stimulate production of endogenous pyrogens, namely cytokines—including IL-1α, IL-1β, TNF-α, IL-6—and interferons, with local and systemic effects. IL-1 stimulates prostaglandin synthesis in hypothalamic thermoregulatory centers, thereby altering the "thermostat" that controls body temperature. Inhibitors of cyclooxygenase (e.g., aspirin) block the fever response by inhibiting IL-1–stimulated PGE_2 synthesis in the hypothalamus. TNF-α and IL-6 also increase body temperature by a direct action on the hypothalamus. Chills (the sensation of cold), rigor (profound chills with shivering and piloerection), and sweats (to allow heat dissipation) are symptoms associated with fever.

Pain

The process of pain is associated with (1) **nociception** (i.e., detection of noxious stimuli and transmission of this information to the brain), (2) pain perception, and (3) suffering and pain behavior. Nociception is primarily a neural response initiated in injured tissues by specific nociceptors, which are high-threshold receptors for thermal, chemical, and mechanical stimuli. Most chemical mediators of inflammation described in this chapter—including ions, kinins, histamine, NO•, prostanoids, cytokines, and growth factors—activate peripheral nociceptors directly or indirectly. Kinins, especially bradykinin, are formed following tissue trauma and in inflammation; they activate primary sensory neurons via B_2 receptors to mediate pain transmission. Another kinin, des-arg bradykinin, activates B_1 receptors to produce pain only during inflammation. Cytokines, particularly TNF-α, IL-1, IL-6 and IL-8, produce pain hypersensitivity to mechanical and thermal stimuli. Prostaglandins and growth factors may directly activate nociceptors but appear to be most important in enhancing nociceptor sensitivity. Pain perception and subsequent behavior arise in response to this enhanced sensitivity to both noxious and normally innocuous stimuli.

Shock

Under conditions of massive tissue injury or infection that spreads to the blood (sepsis), significant quantities of cytokines, especially TNF-α, and other chemical mediators of inflammation may be generated in the circulation. The sustained presence of these mediators induces cardiovascular decompensation through their effects on the heart and on the peripheral vascular system,. Systemic effects include generalized vasodilation, increased vascular permeability, intravascular volume loss, and myocardial depression with decreased cardiac output (SIRS) (see Chapter 7). In severe cases, activation of coagulation pathways may generate microthrombi throughout the body, with consumption of clotting components and subsequent predisposition to bleeding, a condition defined as **disseminated intravascular coagulation** (see Chapter. 20). The net result is **multisystem organ dysfunction** and death.