2 Inflammation

Hedwig S. Murphy

Inflammation is the response to injury of a tissue and its microcirculation and is characterized by elaboration of inflammatory mediators as well as movement of fluid and leukocytes from the blood into extravascular tissues. Inflammation localizes and eliminates microorganisms, damaged cells, and foreign particles, paving the way for a return to normal structure and function.

*The clinical signs of inflammation, recognized in Egyptian medical texts before 1000 BC, were codified as the four cardinal signs of inflammation: **rubor** (redness), **calor** (heat), **tumor** (swelling), and **dolor** (pain) by the Roman encyclopedist Aulus Celsus in the second century AD. These features correspond to inflammatory events of vasodilation, edema, and tissue damage. A fifth sign **functio laesa** (loss of function) was added in the 19th century by Rudolf Virchow, who recognized inflammation as a response to tissue injury.*

Overview Of Inflammation

Inflammation is best viewed as an ongoing process that can be divided into phases.

- **Initiation** results in a stereotypic, immediate response termed **acute inflammation**. The acute response is
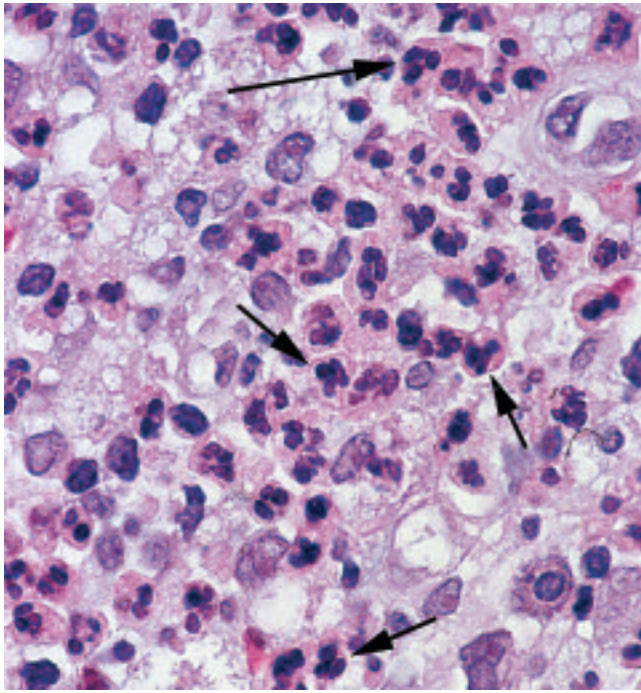
FIGURE 2-1. Acute inflammation with densely packed polymorphonuclear neutrophils (PMNs) with multilobed nuclei (*arrows*).

FIGURE 2-2. Chronic inflammation. Lymphocytes, plasma cells (*arrows*), and a few macrophages are present.

characterized by the rapid flooding of the injured tissue with fluid, coagulation factors, cytokines, chemokines, platelets and inflammatory cells, and neutrophils in particular (Fig. 2-1).

- **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 inciting agent by phagocytosis and enzymatic or nonenzymatic processes reduces or eliminates foreign material or infectious organisms. At the same time, damaged tissue components are also removed, 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 and allow for either restoration of tissue, with return to normal physiological function, or repair and the development of scar in place of normal tissue.

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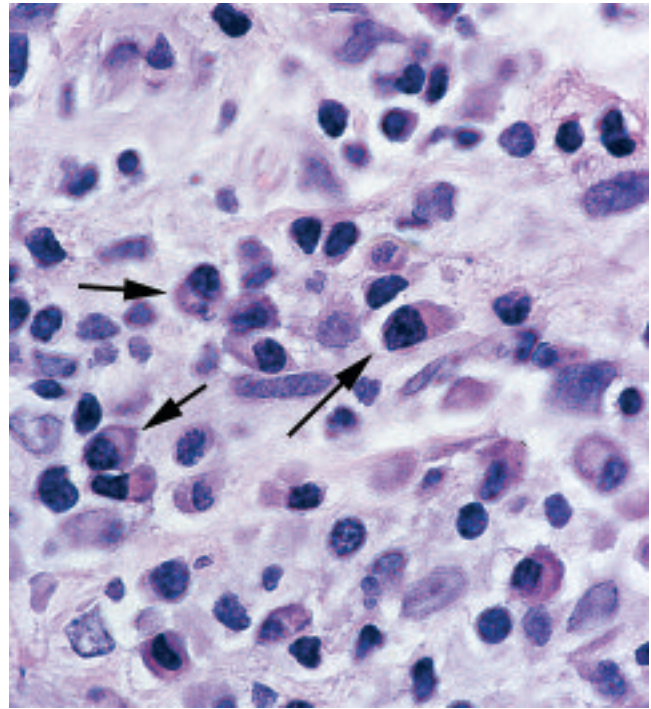
Acute Inflammation: Vascular Events

Among the earliest responses to tissue injury are alterations in the anatomy and function of the microvasculature, which may promote edema (see Figs. 2-3 and 2-4). These responses include:

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.
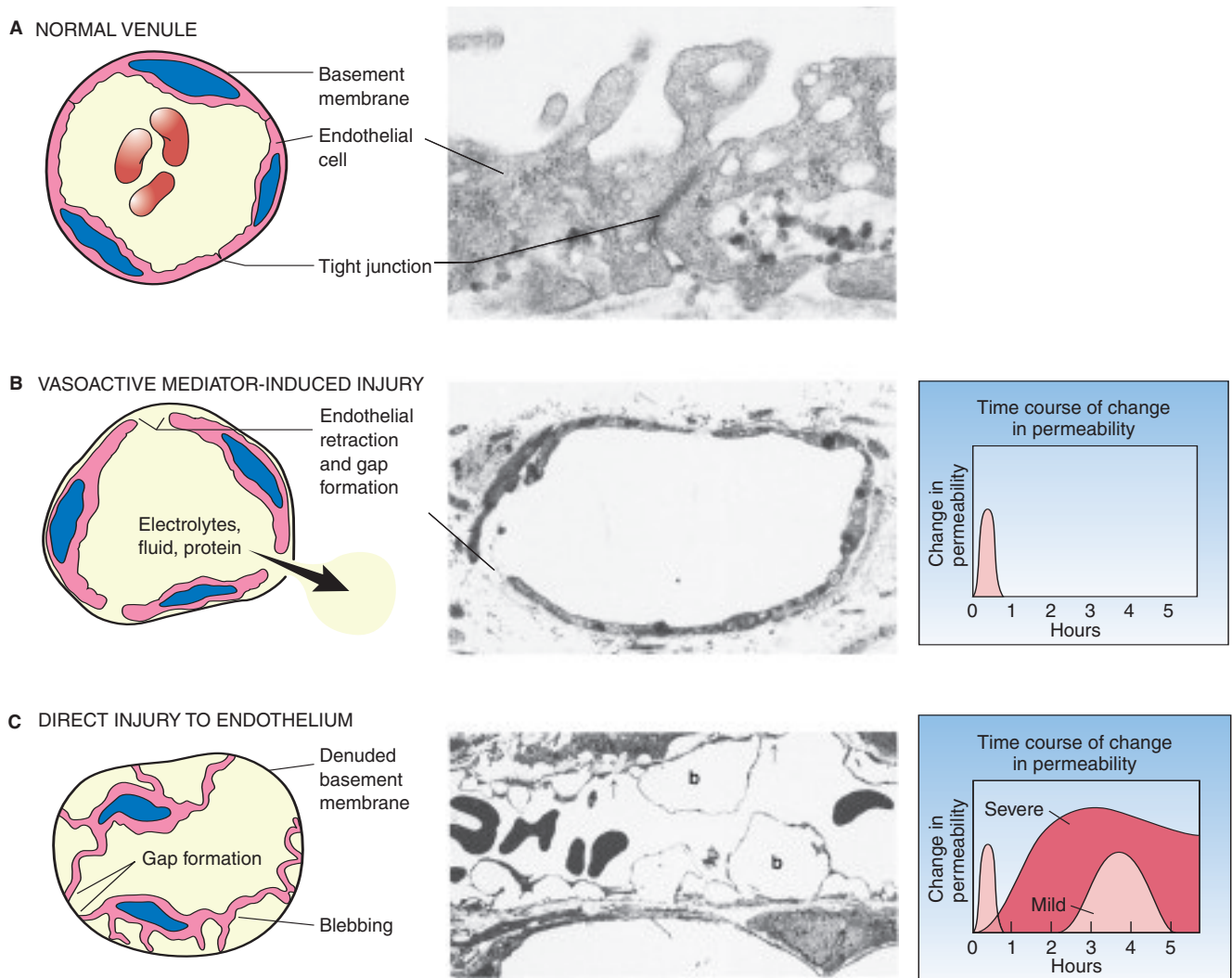
A NORMAL VENULE

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

Change in permeability

0 1 2 3 4 5
Hours

C DIRECT INJURY TO ENDOTHELIUM

Denuded basement membrane

Gap formation

Blebbing

Time course of change in permeability

Change in permeability

Severe

Mild

0 1 2 3 4 5
Hours

FIGURE 2-3. **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.

Vasoactive mediators, originating from both plasma and cellular sources, are generated at sites of tissue injury (see Fig. 2-4). These mediators bind to specific receptors on vascular endothelial and smooth muscle cells, causing vasoconstriction or vasodilation. Proximal to capillaries, vasodilation of arterioles increases blood flow and can exacerbate fluid leakage into the tissue. Distally, vasoconstriction of postcapillary venules increases capillary bed hydrostatic pressure, potentiating edema formation. By contrast, 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. 2-3B). 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. 2-3C), thereby disrupting the barrier between the intravascular and extravascular spaces.
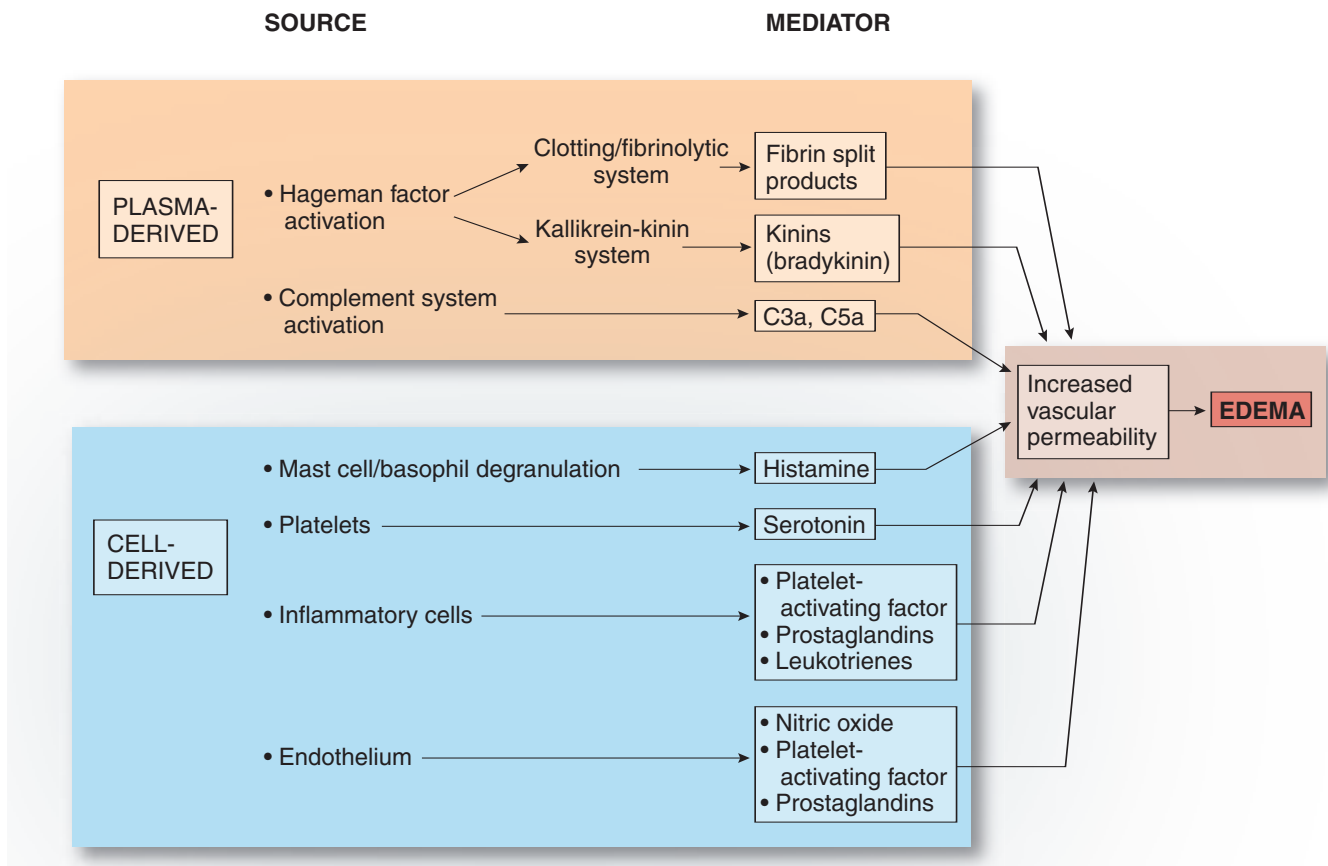
SOURCE   MEDIATOR

FIGURE 2-4. **Inflammatory mediators of** increased vascular permeability.

Several definitions are important for understanding the vascular components of inflammation:

- **Edema** is accumulation of fluid within the extravascular compartment and interstitial tissues.
- A **transudate** is edema fluid with low protein content (specific gravity <1.015). Transudates tend to occur in noninflammatory conditions, where the endothelial barrier remains intact and prevents the loss of large molecules from the vasculature.
- 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 **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 pericardium, it is referred to as "fibrinous pleuritis" or "fibrinous pericarditis."
- **A purulent exudate or effusion** contains prominent cellular components. It is frequently associated with pathological conditions such as pyogenic bacterial infections,

in which the predominant cell type is the polymorphonuclear neutrophil (PMN).

Plasma-Derived Mediators Of Inflammation

Numerous chemical mediators are integral to initiation, amplification, and termination of inflammatory processes (Fig. 2-4). Cell- and plasma-derived mediators work in concert to activate cells by (1) binding specific receptors, (2) recruiting cells to sites of injury, and (3) stimulating the 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. 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 and fibrinolytic system**, (2) **kinin**
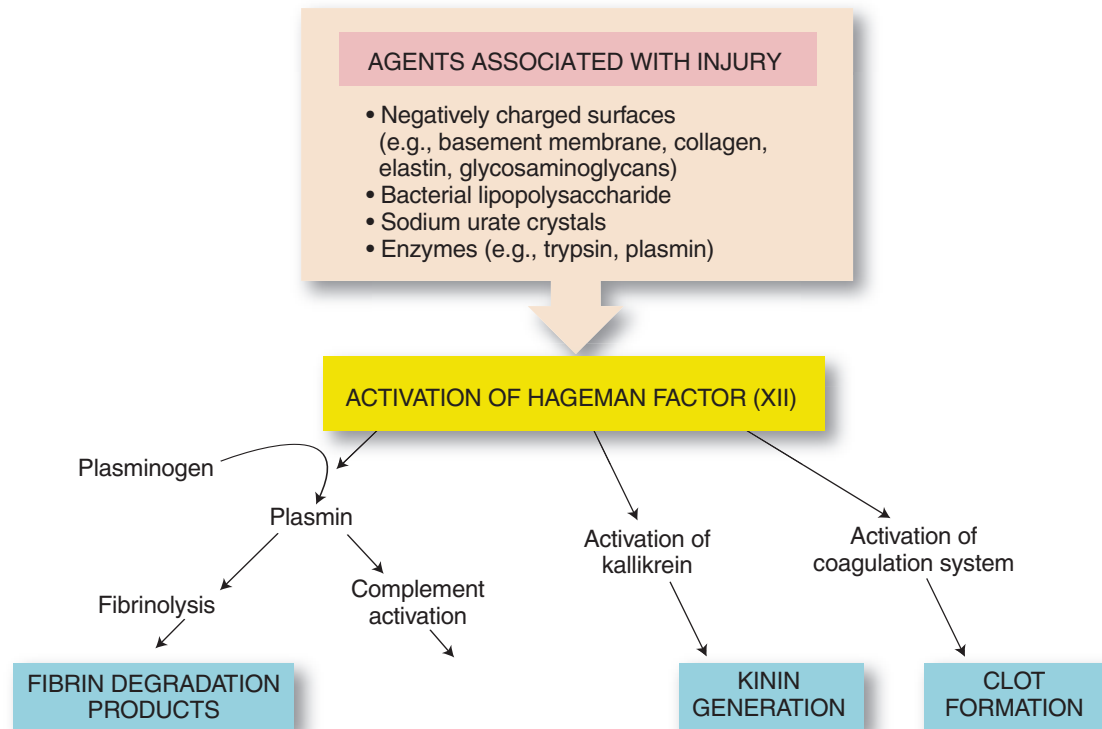
FIGURE 2-5. **Hageman factor activation and inflammatory mediator production.** Hageman factor activation is a key event leading to conversion of plasminogen to plasmin, resulting in generation of fibrin split products and active complement products. Activation of kallikrein produces kinins, and activation of the coagulation system results in clot forma-

generation, and (3) the **complement system** (Fig. 2-5). 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) is generated within the plasma and is activated by exposure to negatively charged surfaces such as basement membranes, proteolytic enzymes, bacterial lipopolysaccharides, and foreign materials. This key component triggers activation of additional plasma protease systems important in inflammation including (1) the "intrinsic" coagulation cascade, (2) fibrinolysis with the concomitant elaboration of plasmin and plasmin-derived bioactive peptides, (3) generation of kallikrein and subsequent production of kinins, and (4) activation of the alternate complement pathway (see Fig. 2-5).

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 extravasation, cell migration, inflammatory cell activation, and inflammatory-mediated pain responses. Kinins 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), and nitric oxide (NO•). Kinins are rapidly degraded to inactive products by kininases and, therefore, have rapid and short-lived functions.

Complement is Activated Through Three Pathways to Form the Membrane Attack Complex (MAC)

The complement system is a group of proteins found in plasma and on cell surfaces, whose primary function is defense against microbes. 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.
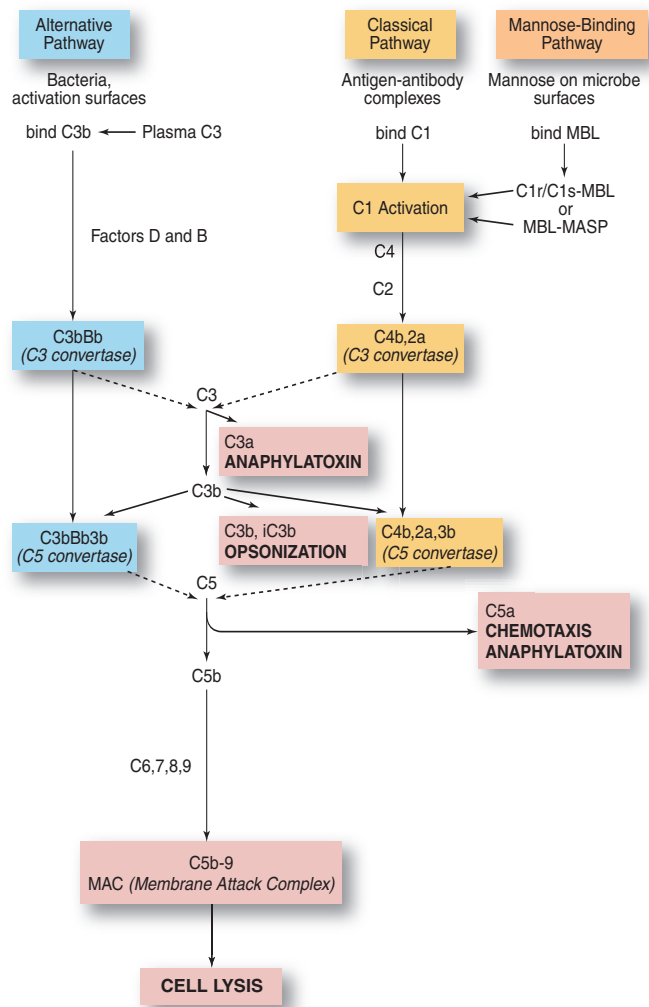
FIGURE 2-6. Complement activation. The alternative, classical, and mannose-binding pathways lead to generation of the complement cascade of inflammatory mediators and cell lysis by the membrane attack complex (MAC). MBL, mannose-binding lectin; MBL-MASP, MBL-associated serine protease.

The endpoint of complement activation is formation of the MAC and cell lysis. The cleavage products generated at each step of the way catalyze the next step in the cascade and have additional properties that render them important inflammatory molecules (Fig. 2-6):

- **Anaphylatoxins** (C3a, C4a, C5a): These proinflammatory molecules mediate smooth-muscle contraction and increase vascular permeability.
- **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 and induce degranulation of mast cells and basophils.

The complement system is activated by three convergent pathways termed **classical**, **mannose-binding lectin (MBL)**, and **alternative pathways** (see Fig. 2-6).

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, which proceeds as follows (see Fig. 2-6).

The Mannose-Binding Pathway

The mannose- or lectin-binding pathway has some components in common with the classical pathway. It is initiated by the binding of microbes bearing terminal mannose groups to **mannose-binding lectin**, a member of the family of calcium-dependent lectins, termed the **collectins**. This multifunctional acute-phase protein has properties similar to those of immunoglobulin 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 (see Fig. 2-6).

Alternative Pathway

The alternative pathway is initiated by derivative products of microorganisms, such as endotoxin (from bacterial cell surfaces), zymosan (yeast cell walls), polysaccharides , viruses, tumor cells, and foreign materials. Proteins of the alternative pathway are called "factors," followed by a letter. Activation of the alternative pathway occurs at the level of C3 activation to produce small amounts of C3b. which become covalently bound to carbohydrates and proteins on microbial cell surfaces (see Fig. 2-6).

The Complement System and Disease

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. Such patients have an increased susceptibility to infectious agents, and in some cases, a propensity for autoimmune diseases associated with circulating immune complexes.

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., NO•).

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. As part of a complex regulatory network, prostanoids, leukotrienes and lipoxin, (derivatives of arachidonic acid) both promote and inhibit inflammation (Table 2-1).

Arachidonic Acid

Depending on the specific inflammatory cell and the nature of the stimulus, activated cells generate arachidonic acid by one of two pathways, involving either stimulus-induced activation of phospholipase A_2 (PLA_2) or phospholipase C. 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. 2-7).

Corticosteroids are widely used to suppress tissue destruction associated with many inflammatory diseases. These drugs 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.

Platelet-Activating Factor (PAF)

Another potent inflammatory mediator derived from membrane phospholipids is PAF, synthesized by virtually all activated inflammatory cells, endothelial cells, and injured tissue cells. PAF is derived from membrane phospholipids by the PLA_2 pathway. During inflammatory and allergic responses, PAF stimulates platelets, neutrophils, monocyte/macrophages, endothelial cells, and vascular

TABLE 2–1	
Biological Activities of Arachidonic Acid Metabolites	
Metabolite	Biological Activity
PGE_2, PGD_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 (especially 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; TXA_2, thromboxane A_2; LT, leukotriene.

smooth muscle cells. PAF induces platelet aggregation and degranulation at sites of tissue injury and enhances release of serotonin, thereby causing changes in vascular permeability. The molecule is also an extremely potent vasodilator, augmenting permeability of microvasculature at sites of tissue injury.

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. 2-7). **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 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 prostacyclins (PGI_2), PGD_2, PGE_2, PGF_2, α 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 (see Table 2-1).
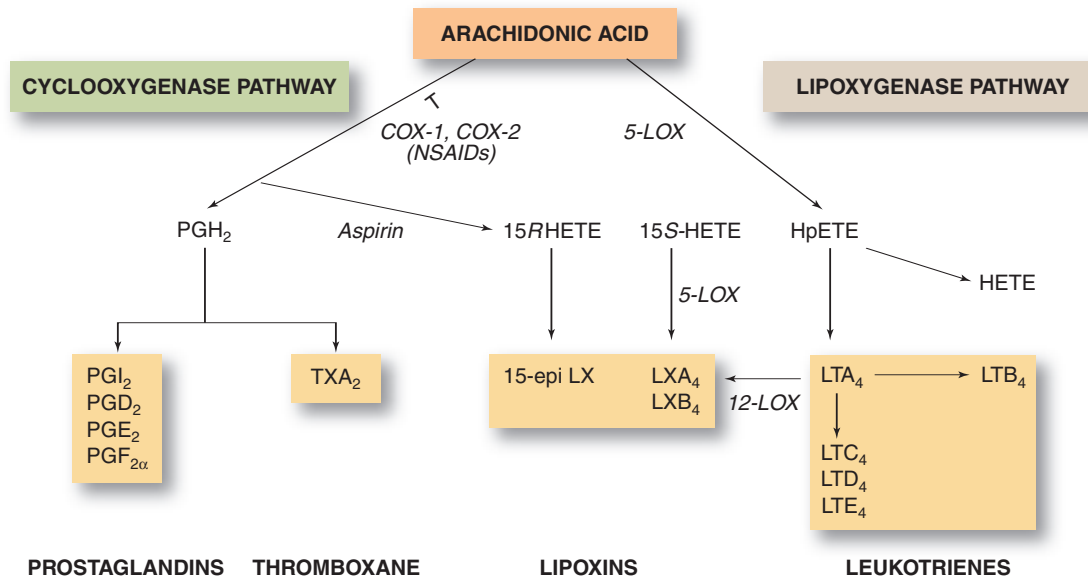
FIGURE 2-7. **Biologically active arachidonic acid metabolites.** The cyclo-oxygenase pathway of arachidonic acid metabolism generates prostaglandins (PG) and thromboxane (TXA_2). The lipoxygenase pathway forms lipoxins (LX) and leukotrienes (LT); COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HpETE, 5-hydroperoxyeicosatetraenoic acid; NSAIDs, nonsteroidal anti-inflammatory drugs.

Inhibition of COX is one mechanism by which nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, indomethacin, and ibuprofen, exert their potent analgesic and anti-inflammatory effects. NSAIDS block COX-2–induced formation of prostaglandins, thereby mitigating pain and inflammation. However, they also inhibit COX-1 and lead to adverse effects on the stomach and kidneys. This complication led to development of COX-2–specific inhibitors (see Fig. 2-7).

Leukotrienes

Slow-reacting substance of anaphylaxis 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. 2-7 and Table 2-1). Leukotriene A_4 (LTA_4) serves as a precursor to several other leukotrienes. LTB_4 is a major product of neutrophils as well as certain macrophage populations and has potent chemotactic activity for neutrophils, monocytes, and macrophages. In other cell types, especially mast cells, basophils and macrophages, LTC_4, LTD_4, and LTE_4 are produced. These three cysteinyl-leukotrienes (1) stimulate smooth-muscle contraction, (2) enhance vascular permeability, and (3) are responsible for the development of many of the clinical symptoms associated with allergic-type reactions, notably 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 proinflammatory products of arachidonic acid, are synthesized by platelets and neutrophils within the vascular lumen in a manner dependent on cell cell interactions (see Fig. 2-7). Neutrophil LTA_4 serves as a source for platelet-dependent synthesis of lipoxins. Monocytes, eosinophils, and airway epithelial cells generate 15S-hydroxyeicosatetraenoic acid (15S-HETE), which is taken up by neutrophils and converted to lipoxins.

Cytokines are Cell-Derived Inflammatory Hormones

Cytokines constitute a group of low-molecular-weight hormone-like proteins secreted by cells. Many cytokines are produced at sites of inflammation, including interleukins, growth factors, colony-stimulating factors, interferons, and chemokines (Fig. 2-8). 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. *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 of endothelial cells and leukocytes (Fig. 2-9). 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).

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 2-8. **Cytokines important in inflammation.** GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; NK, natural killer; IFN, interferon; TFN, tumor necrosis factor.

Interleukins

IL-1 and TNF-α, produced by macrophages, as well as other cells, are central to the 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. 2-9). 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).

Chemokine Structure and Function

Chemokines direct cell migration (a process termed **chemotaxis**), The accumulation of inflammatory cells at sites of tissue injury requires their migration from the vascular space into extravascular tissue. Chemokines are a large class of cytokines (over 50 known members) that regulate leukocyte trafficking in inflammation and immunity. For example, chemokines are important chemotactic factors for PMNs in acute inflammation (see later).

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, namely 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. **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).

Chemokines function as immobilized or soluble molecules that generate a chemotactic gradient by binding to proteoglycans of the extracellular matrix 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 tend to move cells along the chemotactic gradient to the site of injury. This process of responding to a matrix-bound chemoattractant is termed **haptotaxis**. 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.
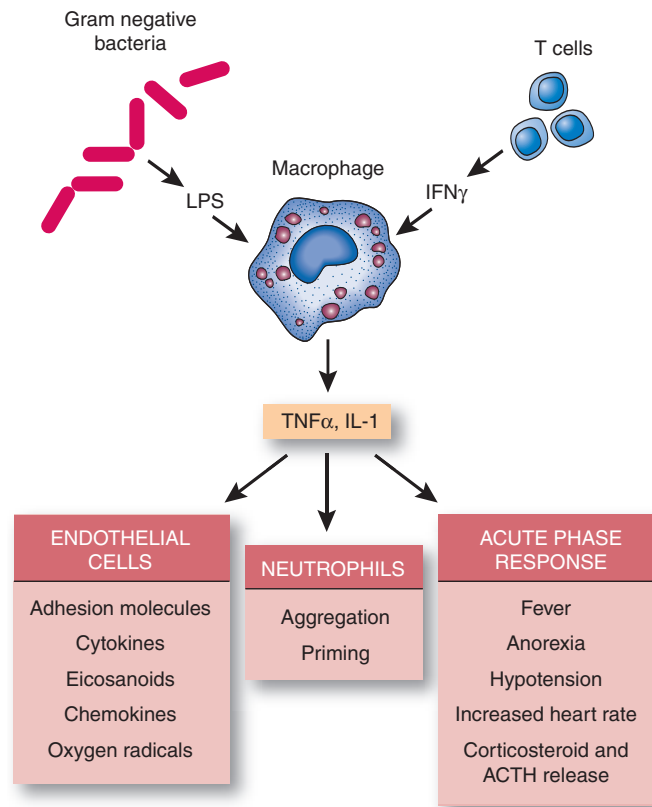
FIGURE 2-9. **Central role of interleukin (IL)-1 and tumor necrosis factor (TNF)-α in inflammation.** Lipopolysaccharide (LPS) and IFN-γ activate macrophages to release inflammatory cytokines, principally IL-1 and TNF-α, responsible for directing local and systemic inflammatory responses. ACTH, adrenocorticotropic hormone.

Reactive Oxygen Species are Signal-Transducing, Bactericidal, and Cytotoxic Molecules

ROS are chemically reactive molecules derived from molecular oxygen. Normally, they are rapidly inactivated, but when generated inappropriately, they can be cytotoxic (see Chapter 1). ROS create **oxidative stress** by activating signal-transduction pathways and combining with proteins, lipids, and DNA. Leukocyte-derived ROS, released within phagosomes, are bactericidal. ROS important in inflammation include superoxide ($O_2\bullet$, O_2^-), $NO\bullet$, hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$) (Fig. 2-10) (see below and Chapter 1).

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 such functions 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.
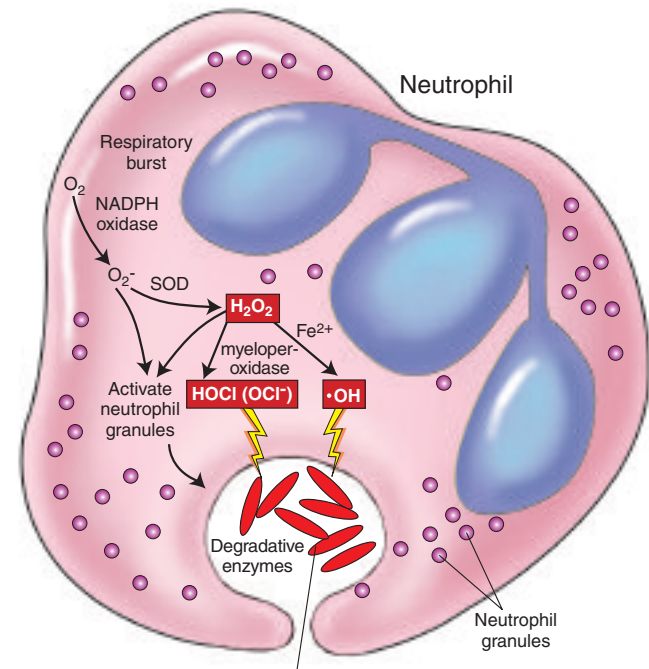

FIGURE 2-10. **Generation of reactive oxygen species** in neutrophils as a result of phagocytosis of bacteria. Fe^{2+}, ferrous iron; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; NADPH, nicotinamide adenine dinucleotide phosphate; OCl^-, hypochlorite radical; $\bullet OH$, hydroxyl radical; SOD, superoxide dismutase.

Neutrophils are the Major Cellular Participant in Acute Inflammation

The PMN is the major cellular participant in acute inflammation. It has granulated cytoplasm and a nucleus with two to four lobes. PMNs are stored in the bone marrow, circulate in the blood, and rapidly accumulate at sites of injury or infection (Fig. 2-11A). They are activated in response to phagocytic stimuli, cytokines, chemotactic mediators or antigen–antibody complexes, which bind specific receptors on their surface membrane. In tissues, PMNs phagocytose invading microbes and dead tissue (see below). Once they are recruited into tissue, they do not re-enter the circulation.

Endothelial Cells Line Blood Vessels

Endothelial cells comprise a monolayer of cells lining blood vessels and help to separate intra- and extravascular spaces. They produce 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. 2-11B).

Endothelial cells are gatekeepers in inflammatory cell recruitment: they can promote or inhibit tissue perfusion and the influx of inflammatory cells. Inflammatory agents, such as bradykinin and histamine, endotoxin and cytokines, in-
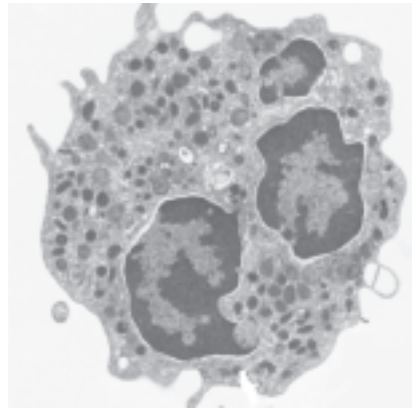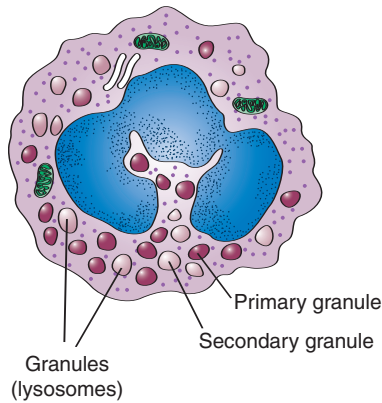
POLYMORPHONUCLEAR LEUKOCYTES

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

Primary granule

Secondary granule

Granules (lysosomes)
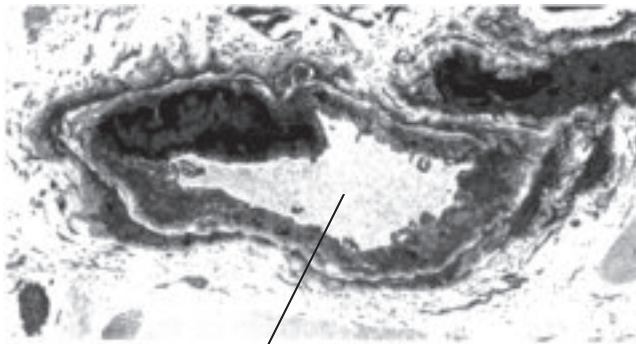
ENDOTHELIAL CELLS

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

FIGURE 2-11. Cells of inflammation: morphology and function. **A.** Neutrophil. **B.** Endothelial cell. Cells of inflammation: morphology and function.

duce endothelial cells to reveal adhesion molecules that (1) anchor and activate leukocytes, (2) present major histocompatibility complex (MHC) class I and II molecules, and (3) generate cytokines and important vasoactive and inflammatory mediators.

Monocyte/Macrophages are Important in Acute and Chronic Inflammation

Circulating monocytes (Fig. 2-11C) 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 where they ingest and process microbes.

Monocyte/macrophages produce potent vasoactive mediators, including prostaglandins and leukotrienes, PAF, and inflammatory cytokines. These cells are especially important for maintaining chronic inflammation.

Mast Cells and Basophils are Important in Allergic Hypersensitivity Reactions

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. 2-11D) 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
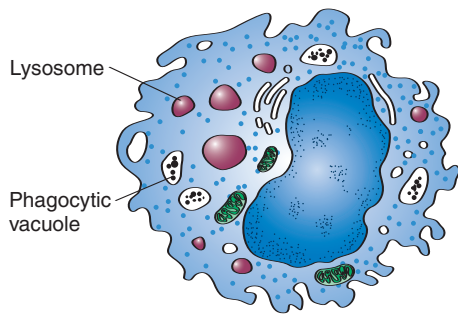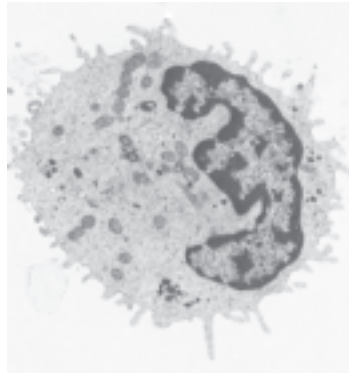
MONOCYTE/MACROPHAGE

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

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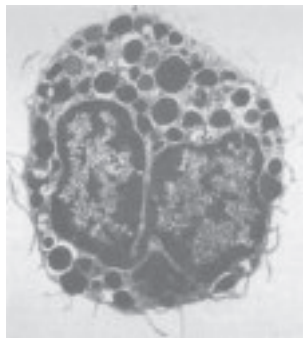- cytokines
  - -IL-1
  - -TNF-α
  - -IL-6
  - -Chemokines (e.g. IL-8, MCP-1)
- lysosomal enzymes
  - -acid hydrolases
  - -serine proteases
  - -metalloproteases (e.g. collagenase)
- cationic proteins
- prostaglandins/leukotrienes
- plasminogen activator
- procoagulant activity
- oxygen metabolite formation

C

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 2-11. *Continued.* **C.** Monocyte/macrophage. **D.** Mast cell. IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF-, tumor necrosis factor-.

microvasculature. Basophils circulate in small numbers and can migrate into tissue.

When IgE-sensitized mast cells or basophils are stimulated by antigens; physical agonists such as cold and trauma, or cationic proteins, inflammatory mediators in the dense cytoplasmic granules are secreted into extracellular tissues. These bodies 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, thereby 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 and cytokines, such as TNF-α and IL-4.

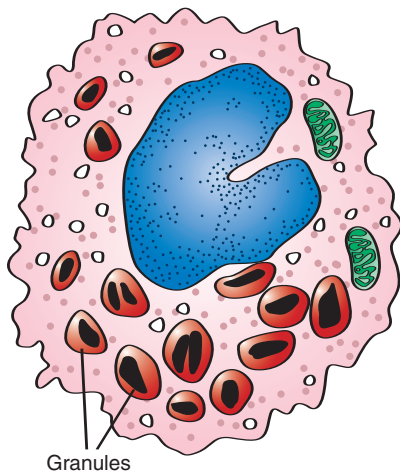## Eosinophils are Important in Defense Against Parasites

Eosinophils circulate in the blood and are recruited to tissue in a manner similar to that of PMNs. They are characteristic of IgE-mediated reactions, such as hypersensitivity, allergic, and asthmatic responses (Fig. 2-12A). Eosinophils contain leukotrienes and PAF, as well as acid phosphatase and peroxidase. They express IgA receptors and exhibit large granules that contain eosinophil major basic protein, both of which are involved in defense against parasites.

Platelets Play a Role in Normal Hemostasis

Platelets play a primary role in normal hemostasis 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. 2-12B). The platelet is small (2 mm in diameter), lacks a nucleus, and contains three distinct kinds of inclusions:

- dense granules, rich in serotonin, histamine, calcium and adenosine diphosphate
- α granules, containing fibrinogen, coagulation proteins, platelet-derived growth factor, and other peptides and proteins
- lysosomes, which sequester acid hydrolases

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

A

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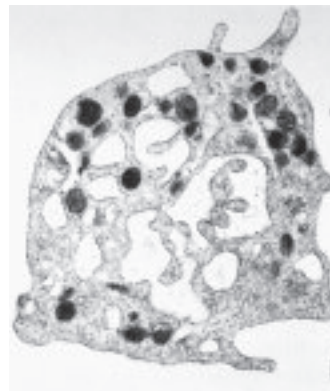**PRIMARY INFLAMMATORY MEDIATORS**
• Dense granules
  -Serotonin
  -Ca^{2+}
  -ADP
• α-granules
  -Cationic proteins
  -Fibrinogen and coagulation proteins
  -Platelet-derived growth factor (PDGF)
• Lysosomes
  -Acid hydrolases
•Thromboxane A_2

B

FIGURE 2-12. More cells of inflammation: morphology and function. **A.** Eosinophil. **B.** Platelet. ADP, adenosine diphosphate.

Platelets adhere, aggregate, and degranulate when they contact fibrillar collagen (e.g., after vascular injury that exposes extracellular matrix [ECM] proteins) or thrombin (after activation of the coagulation system).

Leukocyte Recruitment In Acute Inflammation

One of the essential features of acute inflammation is accumulation of leukocytes, particularly PMNs, in affected tissues. Leukocytes adhere to vascular endothelium, where they become activated. They then flatten and migrate from the vasculature through the endothelial cell layer into surrounding tissue. In the extravascular tissue, PMNs ingest foreign material, microbes, and dead tissue.

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. 2-13). 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 on the endothelium (leukocyte **arrest**). As endothelial cells separate, leukocytes **transmigrate** through the vessel wall and, under the influence of chemotactic
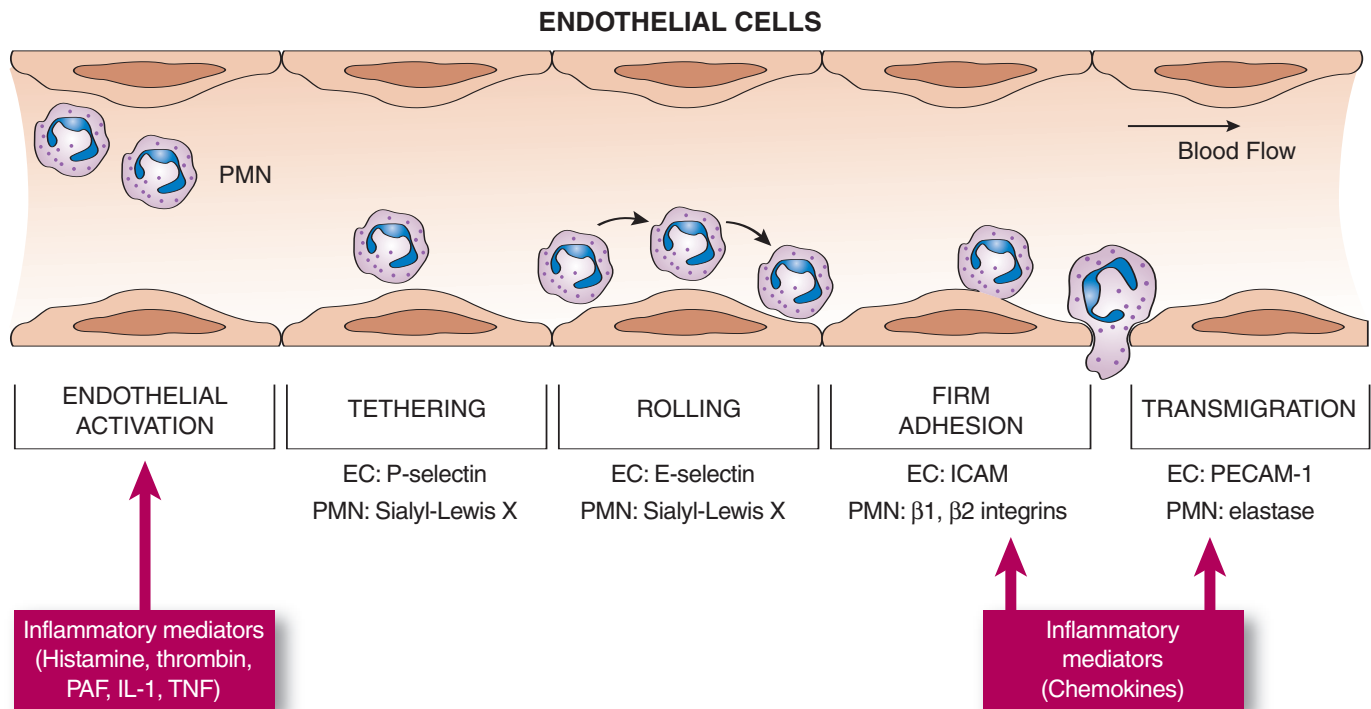
ENDOTHELIAL CELLS

ENDOTHELIAL ACTIVATION	TETHERING	ROLLING	FIRM ADHESION	TRANSMIGRATION
	EC: P-selectin	EC: E-selectin	EC: ICAM	EC: PECAM-1
	PMN: Sialyl-Lewis X	PMN: Sialyl-Lewis X	PMN: β1, β2 integrins	PMN: elastase

Inflammatory mediators (Histamine, thrombin, PAF, IL-1, TNF)

Inflammatory mediators (Chemokines)

FIGURE 2-13. **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.

factors, leukocytes migrate through extravascular tissue to the site of injury.

The events involved in leukocyte recruitment are regulated as follows: (1) Inflammatory mediators stimulate resident tissue cells, including vascular endothelial cells; (2) Adhesion molecules are expressed on vascular endothelial cell surfaces and bind to reciprocal molecules on the surfaces of circulating leukocytes; and (3) Chemotactic factors 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.

Selectins

The selectin family (part of the C-type, calcium-dependent lectin group) 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 carbohydrate-binding domain specific for sialylated oligosaccharides. The last is the sialyl-Lewis X

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 molecules that contain Lewis X.

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 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-selectin ligand 1 (ESL-1), and CD34. They possess sialyl-Lewis X, which binds the lectin domain of selectins. Addressins are expressed at leukocyte and endothelial surfaces. They regulate localization of subpopulations of leukocytes and are involved in lymphocyte activation.

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. Very late activation (VLA) molecules include VLA-4 ($\alpha 4\beta 1$) on leukocytes and lymphocytes that bind VCAM-1 (an immunoglobulin-domain-bearing molecule) on endothelial cells. The $\beta 2$ integrins (CD18) 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 CR3, CD11b/CD18 or Mac-1) bind to both ICAM-1 and ICAM-2 (also members of the Ig domain-bearing family, see below). Leukocyte integrins exist in a low-affinity state, but are converted to a high-affinity state when these cells are activated.

Immunoglobulin Superfamily

Adhesion molecules of the immunoglobulin (Ig) 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 retards 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 (see Fig. 2-13).

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 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 toward the target tissue, down a gradient of one chemoattractant in response to a second more distal chemoattractant gradient. During migration, the cell extends a pseudopod toward 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. Neutrophils must integrate the various signals to arrive at the appropriate site at the correct time to perform their assigned tasks. 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)
- Products of arachidonic acid metabolism, especially LTB_4
- Chemokines

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 particular 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. Vascular

endothelial cells are connected by tight junctions and adherens junctions. CD31 (platelet endothelial cell adhesion molecule) 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. Neutrophils also induce increases in intracellular calcium in endothelial cells, to which the endothelial cells 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.

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 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. 2-14). 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 on bacterial surfaces causes Fcγ receptors on phagocytes to cluster. Subsequent phosphorylation of immunoreceptor tyrosine-based activation motifs, located in the cytosolic domain or γ subunit of the receptor, triggers intracellular signaling events. Tyrosine kinases that associate with the Fcγ receptor are required for signaling during phagocytosis.

3. **Internalization:** In the case of phagocytosis initiated via the Fcγ receptor or the CR3 (CD11b/CD18 receptor), actin assembly occurs directly under the phagocytosed target. Polymerized actin filaments push the plasma membrane forward. The 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 Fig. 2-14).

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 Debridement

Although PMNs are critical for degrading microbes and cell debris, they also contribute to tissue injury. The release of PMN granules at sites of injury is a double-edged sword. On the one hand, debridement 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.

Neutrophil Granules

The armamentarium of enzymes required for degradation of microbes and tissue is generated and contained within PMN cytoplasmic granules. Primary, secondary, and tertiary granules in neutrophils are differentiated morphologically and biochemically: each granule has a unique spectrum of enzymes (see Fig. 2-11A).

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.
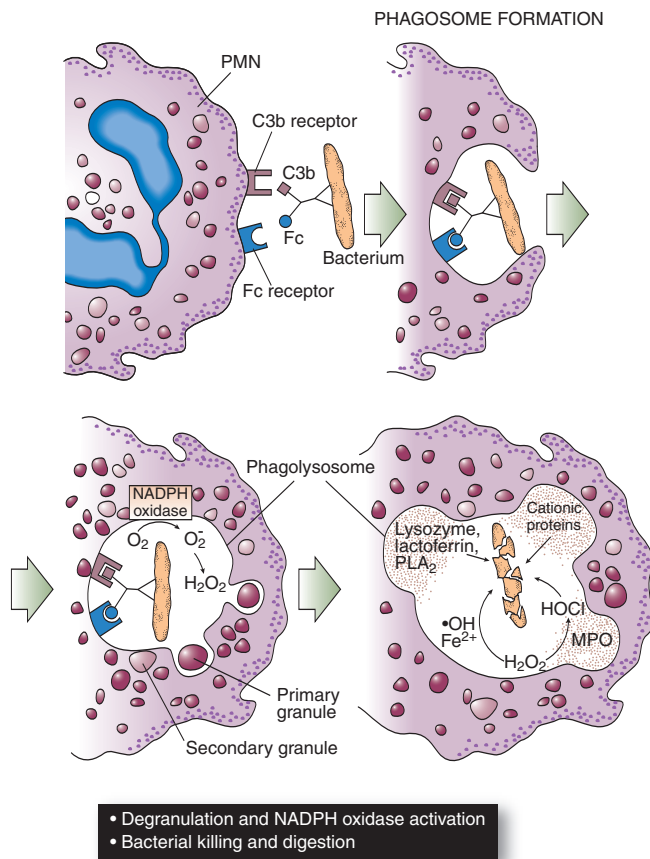
PHAGOSOME FORMATION

FIGURE 2-14. **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.

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. 2-14).

- **Superoxide Anion** (O_2^-): Phagocytosis activates a nicotinamide adenine dinucleotide phosphate (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.**

- **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 strong cationic charge, is secreted from granules during exocytosis. In the presence of a halide, usually chlorine, MPO 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. At the same time, HOCl inactivates α_1-antitrypsin.

- **(•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. Further reduction of •OH leads to formation of H_2O (see Chapter 1).

- **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. It can also 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 as well as 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 by the fungal pathogen *Candida* (Table 2-2).

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

TABLE 2–2	
Congenital Diseases of Defective Phagocytic Cell Function Characterized by Recurrent Bacterial Infections	
Disease	**Defect**
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

LAD, leukocyte adhesion deficiency; NADPH, nicotinamide adenine dinucleotide phosphate; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; Ig, immunoglobulin.

contain hydrolases, including sulfatases, phosphatases, and other enzymes capable of digesting polysaccharides and DNA.

- **Bactericidal/permeability-increasing protein:** 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.
- **Defensins:** Primary granules of PMNs and lysosomes of some mononuclear phagocytes contain this family of cationic proteins, which kill an extensive variety of gram-positive and gram-negative bacteria, fungi, and some enveloped viruses.
- **Lactoferrin:** Lactoferrin is an iron-binding glycoprotein 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 and eosinophilic cationic protein. Major basic protein 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 2-2 shows representative examples of congenital diseases linked to defective phagocytic function.

Outcomes Of Acute Inflammation

As a result of regulatory components and the short life span of neutrophils, acute inflammatory reactions are usually self-limited and are followed by restoration of normal tissue architecture and physiological function **(resolution)**. Resolution involves removal of dead cells, clearance of acute response cells, and re-establishment of the stroma. However, inflammatory responses can lead to other outcomes:

- **Scar:** If a tissue is irreversibly injured, the 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 hyperplasia of lymphoid follicles 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 to infectious agents, including bacteria, viruses and notably parasites, cannot eliminate the organism, infection may persist. Chronic inflammation may also be associated with a variety of noninfectious disease states including:

- **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 from Both the Circulation and Affected Tissue Play a Role in Chronic Inflammation

Monocyte/macrophages, lymphocytes, and plasma cells (see Chapter 4) and cells discussed previously under Acute Inflammation recruited from circulation as well as cells from the affected tissue including fibroblasts and vascular endothelial cells (see Chapter 3) play an active role in chronic inflammation.

Monocyte/Macrophages
Activated macrophages and their cytokines are central to initiating inflammation and prolonging responses that lead to chronic inflammation. (see Fig. 2-11C). 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 the proliferation and activities of fibroblast and endothelial cells.

The **mononuclear phagocyte system** includes blood monocytes, different types of tissue histiocytes and macrophages, particularly Kupffer cells of the liver. Under the influence of chemotactic stimuli, IFN-γ and bacterial endotoxins, resident tissue macrophages are activated and proliferate, while circulating monocytes are recruited and differentiate into tissue macrophages (Fig. 2-15).
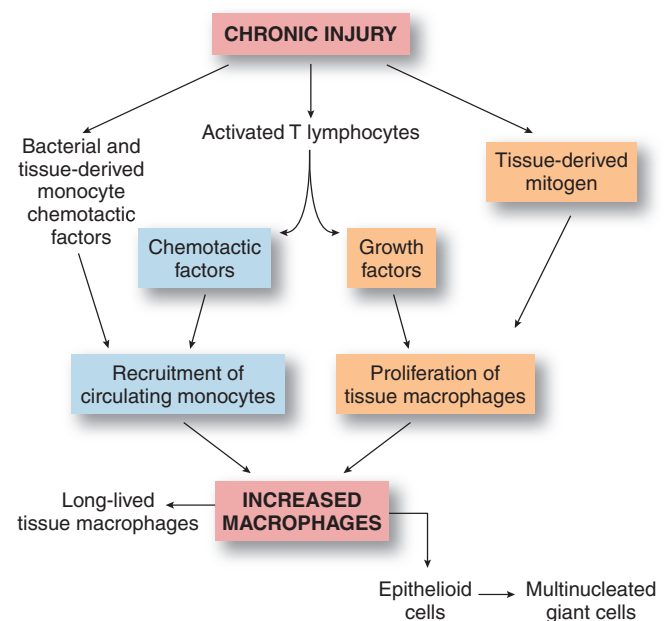

FIGURE 2-15. Accumulation of macrophages in chronic inflammation.

Within different tissues, resident macrophages differ in their armamentarium of enzymes and can respond to local inflammatory signals. The activity of these enzymes is central to the tissue destruction in chronic inflammation. In emphysema, for example, resident macrophages generate proteinases, particularly matrix metalloproteinases (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 and Plasma Cells

Lymphocytes and plasma cells play a central role in the adaptive immune response to pathogens and foreign agents in damaged tissue and are discussed in detail in Chapter 4.

Fibroblasts

Fibroblasts are long-lived, ubiquitous cells whose chief function is to produce components of the ECM (Fig. 2-16). They can also differentiate into other connective tissue cells, including chondrocytes, adipocytes, osteocytes, and smooth muscle cells. Fibroblasts are the construction workers of the tissue, rebuilding the scaffold of ECM upon which tissue is re-established.

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. Fibroblasts function in wound healing in combination with regenerating vascular endothelial cells. Both are 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. Neutrophil products, such as proteases and ROS, protect the host by participating in antimicrobial defense and debridement of damaged tissue; however, these same products may prolong tissue damage and promote chronic inflammation if not appropriately regulated. 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.

Granulomatous Inflammation

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). It prevents dissemination and restricts inflammation due to exogenous substances that are not effectively digested during the acute response, thereby protecting the host tissues. Some autoimmune diseases (e.g., rheumatoid arthritis, Crohn disease, and sarcoidosis
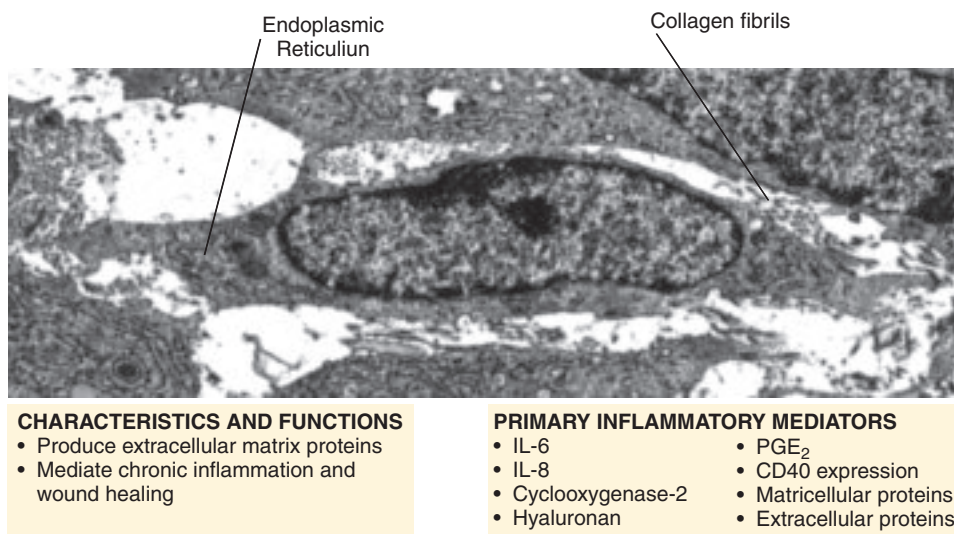
Endoplasmic Reticuliun

Collagen fibrils

CHARACTERISTICS AND FUNCTIONS
- Produce extracellular matrix proteins
- Mediate chronic inflammation and wound healing

PRIMARY INFLAMMATORY MEDIATORS
- IL-6
- IL-8
- Cyclooxygenase-2
- Hyaluronan
- PGE_2
- CD40 expression
- Matricellular proteins
- Extracellular proteins

FIGURE 2-16. Fibroblast: Morphology and function. IL, interleukin.

[a mysterious disease of unknown etiology]) are also associated with granulomas.

The principal cells involved in granulomatous inflammation are macrophages and lymphocytes. 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 transformation into nodular collections of pale, epithelioid cells, creating a **granuloma** (Fig. 2-17A, B). 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**. If a foreign agent (e.g., silica or a *Histoplasma spore*) or other indigestible material is identified within the cytoplasm of a multinucleated giant cell, it is termed a **foreign body giant cell**. Granulomas are further classified histopathologically by the presence or absence of necrosis. Certain infectious agents such as *Mycobacterium tuberculosis* characteristically produce **caseating granulomas**, the necrotic 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.

# Systemic Manifestations Of Inflammation

Under certain conditions, local injury may result in prominent systemic effects that can themselves be debilitating. For example, systemic effects are likely to result when a pathogen enters the bloodstream, a condition known as **sepsis**. The systemic symptoms associated with inflammation, including fever, myalgia, arthralgia, anorexia and somnolence, are attributable to cytokines, including IL-1α, IL-1β, TNF-α, IL-6, and interferons. The most prominent systemic manifestations of inflammation, termed the **systemic inflammatory response syndrome**, are leukocytosis or the acute phase response, fever and shock.

The Acute Phase Response is a Systemic Response to Elevated Levels of IL-1, IL-6, and TNF-α

The acute phase response is a regulated physiological reaction that occurs in inflammatory conditions in response to elevated levels of IL-1, IL-6, and TNF-α. It is characterized clinically by fever, leukocytosis, decreased appetite, and altered sleep patterns and notably by changes in plasma levels of certain *acute phase proteins*. These proteins (Table 2-3) are synthesized primarily by the liver and released in elevated amounts into the circulation where they
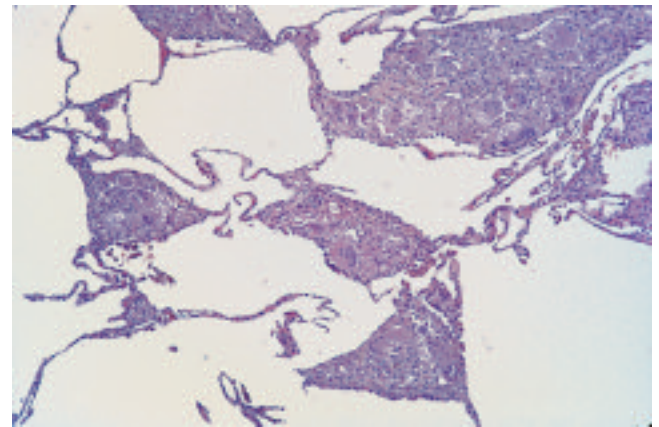
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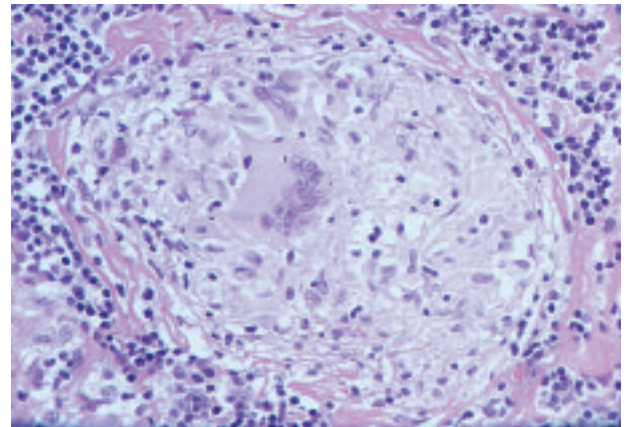
B

FIGURE 2-17. 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.

may serve as markers for ongoing inflammation. For example, increases in acute phase proteins lead to the accelerated erythrocyte sedimentation rate, a qualitative index used clinically to monitor the activity of many inflammatory diseases.

Fever is a Clinical Hallmark of Inflammation

Fever is a clinical hallmark of inflammation. Release of **pyrogens** (molecules that cause fever) by bacteria, viruses, or injured cells may directly affect hypothalamic thermoregulation. More importantly, they stimulate the production of **endogenous pyrogens**, namely cytokines— including IL-1α, IL-1β, TNF-α, IL-6—, and interferons, which produce 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 synthesis

TABLE 2–3

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

of PGE_2. Chills (the sensation of cold), rigor (profound chills with shivering and piloerection), and sweats (to allow heat dissipation) are symptoms associated with fever.

Shock is Characterized by Cardiac Decompensation

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 its effects on the heart and on the peripheral vascular system, a process termed **shock**. Systemic effects include generalized vasodilation, increased vascular permeability, intravascular volume loss, myocardial depression with decreased cardiac output, and potentially death (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.