Acute Inflammation

Acute inflammation is a rapid response to an injurious agent that serves to deliver mediators of host defense—leukocytes and plasma proteins—to the site of injury. Acute inflammation has three major components: (1) alterations in vascular caliber that lead to an increase in blood flow; (2) structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation; and (3) emigration of the leukocytes from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent (Fig. 2-2).

![Diagram of acute inflammation](image-url)

**Figure 2-2** The major local manifestations of acute inflammation, compared to normal. (1) Vascular dilation and increased blood flow (causing erythema and warmth), (2) extravasation and deposition of plasma fluid and proteins (edema), and (3) leukocyte emigration and accumulation in the site of injury.
Certain terms must be defined before specific features of inflammation are described. The escape of fluid, proteins, and blood cells from the vascular system into the interstitial tissue or body cavities is known as exudation. An exudate is an inflammatory extravascular fluid that has a high protein concentration, cellular debris, and a specific gravity above 1.020. It implies significant alteration in the normal permeability of small blood vessels in the area of injury. In contrast, a transudate is a fluid with low protein content (most of which is albumin) and a specific gravity of less than 1.012. It is essentially an ultrafiltrate of blood plasma that results from osmotic or hydrostatic imbalance across the vessel wall without an increase in vascular permeability. Edema denotes an excess of fluid in the interstitial or serous cavities; it can be either an exudate or a transudate. Pus, a purulent exudate, is an inflammatory exudate rich in leukocytes (mostly neutrophils), the debris of dead cells and, in many cases, microbes.

STIMULI FOR ACUTE INFLAMMATION

Acute inflammatory reactions are triggered by a variety of stimuli:

- Infections (bacterial, viral, parasitic) and microbial toxins
- Trauma (blunt and penetrating)
- Physical and chemical agents (thermal injury, e.g., burns or frostbite; irradiation; some environmental chemicals)
- Tissue necrosis (from any cause)
- Foreign bodies (splinters, dirt, sutures)
- Immune reactions (also called hypersensitivity reactions)

Each of these stimuli may induce reactions with some distinctive features, but all inflammatory reactions share the same basic features. We first describe the characteristic reactions of acute inflammation, and then the chemical mediators responsible for these reactions.

VASCULAR CHANGES

Since the two major mechanisms of host defense against microbes—antibodies and leukocytes—are normally carried in the bloodstream, it is not surprising that vascular phenomena play a major role in acute inflammation. Normally, plasma proteins and circulating cells are sequestered inside the vessels and move in the direction of flow. In inflammation, blood vessels undergo a series of changes that are designed to maximize the movement of plasma proteins and circulating cells out of the circulation and into the site of injury or infection.

Changes in Vascular Flow and Caliber

Changes in vascular flow and caliber begin early after injury and develop at varying rates depending on the severity of the injury. The changes occur in the following order:
Vasodilation is one of the earliest manifestations of acute inflammation; sometimes, it follows a transient constriction of arterioles, lasting a few seconds. Vasodilation first involves the arterioles and then results in opening of new capillary beds in the area. Thus comes about increased blood flow, which is the cause of the heat and the redness (see Fig. 2-2). Vasodilation is induced by the action of several mediators, notably histamine and nitric oxide, on vascular smooth muscle; these mediators are described later in the chapter.

Vasodilation is quickly followed by increased permeability of the microvasculature, with the outpouring of protein-rich fluid into the extravascular tissues; this process is described in detail below.

The loss of fluid results in concentration of red cells in small vessels and increased viscosity of the blood, reflected by the presence of dilated small vessels packed with red cells and slower blood flow, a condition termed stasis. With mild stimuli, stasis may not become apparent until 15 to 30 minutes have elapsed, whereas with severe injury, stasis may occur in a few minutes.

As stasis develops, leukocytes, principally neutrophils, accumulate along the vascular endothelium. Leukocytes then stick to the endothelium, and soon afterward they migrate through the vascular wall into the interstitial tissue, in processes that are described later.

Increased Vascular Permeability (Vascular Leakage)

A hallmark of acute inflammation is increased vascular permeability leading to the escape of a protein-rich fluid (exudate) into the extravascular tissue. The loss of protein from the plasma reduces the intravascular osmotic pressure and increases the osmotic pressure of the interstitial fluid. Together with the increased hydrostatic pressure owing to increased blood flow through the dilated vessels, this leads to a marked outflow of fluid and its accumulation in the interstitial tissue (Fig. 2-3). The net increase of extravascular fluid results in edema.
Figure 2-3  Blood pressure and plasma colloid osmotic forces in normal and inflamed microcirculation. A, Normal hydrostatic pressure (red arrows) is about 32 mm Hg at the arterial end of a capillary bed and 12 mm Hg at the venous end; the mean colloid osmotic pressure of tissues is approximately 25 mm Hg (green arrows), which is equal to the mean capillary pressure. Although fluid tends to leave the precapillary arteriole, it is returned in equal amounts via the postcapillary venule, so that the net flow (black arrows) in or out is zero. B, Acute inflammation. Arteriole pressure is increased to 50 mm Hg, the mean capillary pressure is increased because of arteriolar dilation, and the venous pressure increases to approximately 30 mm Hg. At the same time, osmotic pressure is reduced (averaging 20 mm Hg) because of protein leakage across the venule. The net result is an excess of extravasated fluid.
Normal fluid exchange and microvascular permeability are critically dependent on an intact endothelium. How then does the endothelium become leaky in inflammation? The following mechanisms have been proposed (Fig. 2-4):

- **Formation of endothelial gaps in venules.** This is the most common mechanism of vascular leakage and is elicited by histamine, bradykinin, leukotrienes, the neuropeptide substance P, and many other classes of chemical mediators. It occurs rapidly after exposure to the mediator and is usually reversible and short-lived (15 to 30 minutes); it is thus known as the *immediate transient response*. Classically, this type of leakage affects venules 20 to 60 µm in diameter, leaving capillaries and arterioles unaffected (Fig. 2-5). The precise reason for this restriction to venules is uncertain; it may be because there is a greater density of receptors for the mediators in venular endothelium. Parenthetically, many of the later leukocyte events in inflammation—adhesion and emigration—also occur predominantly in the venules in most organs. Binding of mediators, such as histamine, to their receptors on endothelial cells activates intracellular signaling pathways that lead to phosphorylation of contractile and cytoskeletal proteins, such as myosin. These proteins contract, leading to contraction of the endothelial cells and separation of intercellular junctions. Thus, the gaps in the venular endothelium are largely intercellular or close to the intercellular junctions. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and interferon-γ (IFN-γ) also increase vascular permeability by inducing a structural reorganization of the cytoskeleton, such that the endothelial cells retract from one another. In contrast to the histamine effect, the cytokine-induced response is somewhat delayed (4 to 6 hours) and long-lived (24 hours or more).

- **Direct endothelial injury, resulting in endothelial cell necrosis and detachment.** This effect is usually encountered in necrotizing injuries and is due to direct damage to the endothelium by the injurious stimulus, as, for example, in severe burns or lytic bacterial infections. Neutrophils that adhere to the endothelium (discussed below) may also injure the endothelial cells. In most instances, leakage starts immediately after injury and is sustained at a high level for several hours until the damaged vessels are thrombosed or repaired. The reaction is known as the *immediate sustained response*. All levels of the microcirculation are affected, including venules, capillaries, and arterioles. Endothelial cell detachment is often associated with platelet adhesion and thrombosis.

- **Delayed prolonged leakage.** This is a curious but relatively common type of increased permeability that begins after a delay of 2 to 12 hours, lasts for several hours or even days, and involves venules as well as capillaries. Such leakage is caused, for example, by mild to moderate thermal injury, x-radiation or ultraviolet radiation, and certain bacterial toxins. Late-appearing sunburn is a good example of a delayed reaction. The mechanism of such leakage is unclear. It may result from the direct effect of the injurious agent, leading to delayed endothelial cell damage (perhaps by apoptosis), or the effect of cytokines causing endothelial retraction, as described earlier.

- **Leukocyte-mediated endothelial injury.** Leukocytes adhere to endothelium relatively early in inflammation. As discussed later, such leukocytes may be activated in the process, releasing toxic oxygen species and proteolytic enzymes, which then cause endothelial injury or
detachment, resulting in increased permeability. In acute inflammation, this form of injury is largely restricted to vascular sites, such as venules and pulmonary and glomerular capillaries, where leukocytes adhere for prolonged periods to the endothelium.\[12\]

> Increased transcytosis across the endothelial cytoplasm. Transcytosis occurs across channels consisting of clusters of interconnected, uncoated vesicles and vacuoles called the vesiculovacuolar organelle, many of which are located close to intercellular junctions. Certain factors, for example, vascular endothelial growth factor (VEGF) (Chapter 3), appear to cause vascular leakage by increasing the number and perhaps the size of these channels.\[13\] It has been claimed that this is also a mechanism of increased permeability induced by histamine and most chemical mediators.

> Leakage from new blood vessels. As described in Chapter 3, during repair, endothelial cells proliferate and form new blood vessels, a process called angiogenesis. New vessel sprouts remain leaky until the endothelial cells mature and form intercellular junctions. In addition, certain factors that cause angiogenesis (e.g., VEGF) also increase vascular permeability,\[14\] and endothelial cells in foci of angiogenesis have increased density of receptors for vasoactive mediators, including histamine, substance P, and VEGF.\[15\] All these factors account for the edema that is characteristic of the early phases of healing that follow inflammation (Chapter 3).

**Figure 2-5** Vascular leakage induced by chemical mediators. A, This is a fixed and cleared preparation of a rat cremaster muscle examined unstained by transillumination. One hour before sacrifice, bradykinin was injected over this muscle, and colloidal carbon was given intravenously. Plasma, loaded with carbon, escaped, but most of the carbon particles were retained by the basement membrane of the leaking vessels, with the result that these became "labeled" black. Note that not all the vessels leak—only the venules. In B, a higher power, the capillary network is faintly visible in the background. (Courtesy of Dr. Guido Majno, University of Massachusetts Medical School, Worcester, MA.)
Figure 2-4  Diagrammatic representation of five mechanisms of increased vascular permeability in inflammation (see text).

Although these mechanisms are separable, all may play a role in response to one stimulus. For example, at different stages of a thermal burn, leakage results from chemically mediated endothelial contraction, direct and leukocyte-dependent endothelial injury, and regenerating capillaries when the injury begins to heal. The vascular leakage induced by all these mechanisms accounts for the life-threatening loss of fluid in severely burned patients.
In summary, in acute inflammation, fluid loss from vessels with increased permeability occurs in distinct phases: (1) an immediate transient response lasting for 30 minutes or less, mediated mainly by the actions of histamine and leukotrienes on endothelium; (2) a delayed response starting at about 2 hours and lasting for about 8 hours, mediated by kinins, complement products, and other factors; and (3) a prolonged response that is most noticeable after direct endothelial injury, for example, after burns.

**CELLULAR EVENTS: LEUKOCYTE EXTRAVASATION AND PHAGOCYTOSIS**

A critical function of inflammation is to deliver leukocytes to the site of injury and to activate the leukocytes to perform their normal functions in host defense. Leukocytes ingest offending agents, kill bacteria and other microbes, and get rid of necrotic tissue and foreign substances. A price that is paid for the defensive potency of leukocytes is that they may induce tissue damage and prolong inflammation, since the leukocyte products that destroy microbes and necrotic tissues can also injure normal host tissues.

*The sequence of events in the journey of leukocytes from the vessel lumen to the interstitial tissue, called extravasation, can be divided into the following steps:*

1. In the lumen: margination, rolling, and adhesion to endothelium. Vascular endothelium normally does not bind circulating cells or impede their passage. In inflammation, the endothelium has to be activated to permit it to bind leukocytes, as a prelude to their exit from the blood vessels.

2. Transmigration across the endothelium (also called diapedesis)

3. Migration in interstitial tissues toward a chemotactic stimulus

*Figure 2-6*  The multistep process of leukocyte migration through blood vessels, shown here for neutrophils. The leukocytes first roll, then become activated and adhere to endothelium, then transmigrate across the endothelium, pierce the basement membrane, and migrate.
toward chemoattractants emanating from the source of injury. Different molecules play predominant roles in different steps of this process—selectins in rolling; chemokines in activating the neutrophils to increase avidity of integrins (in green); integrins in firm adhesion; and CD31 (PECAM-1) in transmigration.

In normally flowing blood in venules, erythrocytes are confined to a central axial column, displacing the leukocytes toward the wall of the vessel. Because blood flow slows early in inflammation (stasis), hemodynamic conditions change (wall shear stress decreases), and more white cells assume a peripheral position along the endothelial surface. This process of leukocyte accumulation is called margination. Subsequently, individual and then rows of leukocytes tumble slowly along the endothelium and adhere transiently (a process called rolling), finally coming to rest at some point where they adhere firmly (resembling pebbles over which a stream runs without disturbing them). In time, the endothelium can be virtually lined by white cells, an appearance called pavementing. After firm adhesion, leukocytes insert pseudopods into the junctions between the endothelial cells, squeeze through interendothelial junctions, and assume a position between the endothelial cell and the basement membrane. Eventually, they traverse the basement membrane and escape into the extravascular space. Neutrophils, monocytes, lymphocytes, eosinophils, and basophils all use the same pathway to migrate from the blood into tissues. We now examine the molecular mechanisms of each of the steps.

Leukocyte Adhesion and Transmigration

Leukocyte adhesion and transmigration are regulated largely by the binding of complementary adhesion molecules on the leukocyte and endothelial surfaces, and chemical mediators—chemoattractants and certain cytokines—affect these processes by modulating the surface expression or avidity of such adhesion molecules. The adhesion receptors involved belong to four molecular families—the selectins, the immunoglobulin superfamily, the integrins, and mucin-like glycoproteins. The most important of these are listed in Table 2-1.

<table>
<thead>
<tr>
<th>Endothelial Molecule</th>
<th>Leukocyte Receptor</th>
<th>Major Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin</td>
<td>Sialyl-Lewis X</td>
<td>Rolling (neutrophils, monocytes, lymphocytes)</td>
</tr>
<tr>
<td></td>
<td>PSGL-1</td>
<td></td>
</tr>
<tr>
<td>E-selectin</td>
<td>Sialyl-Lewis X</td>
<td>Rolling, adhesion to activated endothelium (neutrophils, monocytes, T cells)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>CD11/CD18 (integrins)</td>
<td>Adhesion, arrest, transmigration (all leukocytes)</td>
</tr>
<tr>
<td></td>
<td>(LFA-1, Mac-1)</td>
<td></td>
</tr>
<tr>
<td>Endothelial Molecule</td>
<td>Leukocyte Receptor</td>
<td>Major Role</td>
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<tr>
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</tr>
<tr>
<td>VCAM-1</td>
<td>α4β1 (VLA4) (integrins)</td>
<td>Adhesion (eosinophils, monocytes, lymphocytes)</td>
</tr>
<tr>
<td></td>
<td>α4β7 (LPAM-1)</td>
<td></td>
</tr>
<tr>
<td>GlyCam-1</td>
<td>L-selection</td>
<td>Lymphocyte homing to high endothelial venules</td>
</tr>
<tr>
<td>CD31 (PECAM)</td>
<td>CD31</td>
<td>Leukocyte migration through endothelium</td>
</tr>
</tbody>
</table>

*ICAM-1, VCAM-1, and CD31 belong to the immunoglobulin family of proteins; PSGL-1, P-selectin glycoprotein ligand 1.

**Selectins**, so called because they are characterized by an extracellular N-terminal domain related to sugar-binding mammalian lectins, consist of E-selectin (CD62E, previously known as ELAM-1), which is confined to endothelium; P-selectin (CD62P, previously called GMP140 or PADGEM), which is present in endothelium and platelets; and L-selectin (CD62L, previously known by many names, including LAM-1), which is expressed on most leukocyte types (Box 2-1).

Selectins bind, through their lectin domain, to sialylated forms of oligosaccharides (e.g., sialylated Lewis X), which themselves are covalently bound to various mucin-like glycoproteins (GlyCAM-1, PSGL-1, ESL-1, and CD34).

**Selectins and Integrins: Adhesion Molecules Involved in the Inflammatory Response**

The specific (nonrandom) adhesion of cells to other cells or to extracellular matrices is a basic component of cell migration and recognition and underlies many biologic processes, including embryogenesis, tissue repair, and immune and inflammatory responses. It is, therefore, not surprising that many different genes have evolved that encode proteins with specific adhesive functions. Two families of adhesive proteins that are especially important in inflammation are the **selectins** and the **integrins**.

**Selectins**

The selectins are a family of three closely related proteins that differ in their cellular distribution but all function in adhesion of leukocytes to endothelial cells. All selectins are single-chain transmembrane glycoproteins with an amino terminus that is related to carbohydrate-binding proteins known as C-type lectins. Like other C-type lectins, ligand binding by selectins is calcium-dependent (hence the name C-type). The binding of selectins to their ligands has a fast on rate but also has a fast off rate and is of low affinity; this property allows selectins to mediate initial attachment and subsequent rolling of leukocytes on endothelium in the face of flowing blood.

*L-selectin*, or CD62L, is expressed on lymphocytes and other leukocytes. It serves as a homing receptor for lymphocytes to enter lymph nodes by binding to high
endothelial venules (HEVs). It also serves to bind neutrophils to cytokine-activated endothelial cells at sites of inflammation. L-selectin is located on the tips of microvillus projections of leukocytes, facilitating its interaction with ligands on endothelium. At least three endothelial cell ligands can bind L-selectin—glycan-bearing cell adhesion molecule-1 (GlyCAM-1), a secreted proteoglycan found on HEVs of lymph node; mucosal addressin cell adhesion molecule-1 (MadCAM-1), expressed on endothelial cells in gut-associated lymphoid tissues; and CD34, a proteoglycan on endothelial cells (and bone marrow cells). The protein backbones of all these ligands are modified by specific carbohydrates, which are the molecules actually recognized by the selectin.

**E-selectin, or CD62E**, previously known as endothelial leukocyte adhesion molecule-1 (ELAM-1), is expressed only on cytokine-activated endothelial cells, hence the designation E. E-selectin recognizes complex sialylated carbohydrate groups related to the Lewis X or Lewis A family found on various surface proteins of granulocytes, monocytes, and previously activated effector and memory T cells. E-selectin is important in the homing of effector and memory T cells to some peripheral sites of inflammation, particularly in the skin. Endothelial cell expression of E-selectin is a hallmark of acute cytokine-mediated inflammation, and antibodies to E-selectin can block leukocyte accumulation in vivo.

**P-selectin (CD62P)** was first identified in the secretory granules of platelets, hence the designation P. It has since been found in secretory granules of endothelial cells, called Weibel-Palade bodies. When endothelial cells or platelets are stimulated, P-selectin is translocated within minutes to the cell surface. On reaching the cell surface, P-selectin mediates binding of neutrophils, T lymphocytes, and monocytes. The complex carbohydrate ligands recognized by P-selectin appear similar to those recognized by E-selectin.

The essential physiologic roles of selectins have been reinforced by studies of gene knockout mice. L-selectin-deficient mice have small, poorly formed lymph nodes with few T cells. Mice lacking either E-selectin or P-selectin have only mild defects in leukocyte recruitment, suggesting that these two molecules are functionally redundant. Double knockout mice lacking both E-selectin and P-selectin have significantly impaired leukocyte recruitment and increased susceptibility to infections. Humans who lack one of the enzymes needed to express the carbohydrate ligands for E-selectin and P-selectin on neutrophils have similar problems, resulting in a syndrome called leukocyte adhesion deficiency-2 (LAD-2) (see text).

**Integrins**

The integrin superfamily consists of about 30 structurally homologous proteins that promote cell-cell or cell-matrix interactions. The name of this family of proteins derives from the hypothesis that they coordinate (i.e., "integrate") signals from extracellular ligands with cytoskeleton-dependent motility, shape change, and...
All integrins are heterodimeric cell surface proteins composed of two noncovalently linked polypeptide chains, α and β. The extracellular domains of the two chains bind to various ligands, including extracellular matrix glycoproteins, activated complement components, and proteins on the surfaces of other cells. Several integrins bind to Arg-Gly-Asp (RGD) sequences in the fibronectin and vitronectin molecules. The cytoplasmic domains of the integrins interact with cytoskeletal components (including vinculin, talin, actin, α-actinin, and tropomyosin).

Three integrin subfamilies were originally defined on the basis of which of three β subunits were used to form the heterodimers. More recently, five additional β chains have been identified. The β1-containing integrins are also called VLA molecules, referring to "very late activation" molecules, because α1β1 and α2β1 were first shown to be expressed on T cells 2 to 4 weeks after repetitive stimulation in vitro. In fact, other VLA integrins are constitutively expressed on some leukocytes and rapidly induced on others. The β1 integrins are also called CD49a-hCD29, CD49a-h referring to different α chains (α1-α8) and CD29 referring to the common β1 subunit. Most of the β1 integrins are widely expressed on leukocytes and other cells and mediate attachment of cells to extracellular matrices. VLA-4 (α4β1) is expressed only on leukocytes and can mediate attachment of these cells to endothelium by interacting with vascular cell adhesion molecule-1 (VCAM-1). VLA-4 is one of the principal surface proteins that mediate homing of lymphocytes to endothelium at peripheral sites of inflammation.

The β2 integrins are also called CD11a-cCD18, or the leukocyte function-associated antigen-1 (LFA-1) family, CD11a-c referring to different α chains and CD18 to the common β2 subunit. LFA-1 (CD11aCD18) plays an important role in the adhesion of lymphocytes and other leukocytes with other cells, such as antigen-presenting cells and vascular endothelium. Other members of the family include CD11bCD18 (Mac-1 or CR3) and CD11cCD18 (p150,95 or CR4), which mediate leukocyte attachment to endothelial cells and subsequent extravasation. CD11bCD18 also functions as a fibrinogen receptor and as a complement receptor on phagocytic cells, binding particles opsonized with a by-product of complement activation called the inactivated C3b (iC3b) fragment.

The other integrins are expressed on platelets and other cell types, and bind to extracellular matrix proteins as well as proteins involved in coagulation.

The immunoglobulin family molecules include two endothelial adhesion molecules: ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1). Both these molecules serve as ligands for integrins found on leukocytes.

Integrins are transmembrane heterodimeric glycoproteins, made up of α and β chains, that are
expressed on many cell types and bind to ligands on endothelial cells, other leukocytes, and the extracellular matrix (Box 2-1).\textsuperscript{20} The $\beta_2$ integrins LFA-1 and Mac-1 (CD11a/CD18 and CD11b/CD18) bind to ICAM-1, and the $\beta_1$ integrins (such as VLA-4) bind VCAM-1.

Mucin-like glycoproteins, such as heparan sulfate, serve as ligands for the leukocyte adhesion molecule called CD44. These glycoproteins are found in the extracellular matrix and on cell surfaces.

The recruitment of leukocytes to sites of injury and infection is a multistep process involving attachment of circulating leukocytes to endothelial cells and their migration through the endothelium (see Fig. 2-6). The first events are the induction of adhesion molecules on endothelial cells, by a number of mechanisms (Fig. 2-7). Mediators such as histamine, thrombin, and platelet activating factor (PAF) stimulate the redistribution of P-selectin from its normal intracellular stores in granules (Weibel-Palade bodies) to the cell surface. Resident tissue macrophages, mast cells, and endothelial cells respond to injurious agents by secreting the cytokines TNF, IL-1, and chemokines (chemoattractant cytokines). (Cytokines are described in more detail below and in Chapter 6.) TNF and IL-1 act on the endothelial cells of postcapillary venules adjacent to the infection and induce the expression of several adhesion molecules. Within 1 to 2 hours, the endothelial cells begin to express E-selectin. Leukocytes express at the tips of their microvilli carbohydrate ligands for the selectins, which bind to the endothelial selectins. These are low-affinity interactions with a fast off-rate, and they are easily disrupted by the flowing blood. As a result, the bound leukocytes detach and bind again, and thus begin to roll along the endothelial surface.
Figure 2-7  Regulation of endothelial and leukocyte adhesion molecules. A, Redistribution of P-selectin. B, Cytokine activation of endothelium. C, Increased binding avidity of integrins (see text).

TNF and IL-1 also induce endothelial expression of ligands for integrins, mainly VCAM-1 (the ligand for the VLA-4 integrin) and ICAM-1 (the ligand for the LFA-1 and Mac-1 integrins). Leukocytes normally express these integrins in a low-affinity state. Meanwhile, chemokines that were produced at the site of injury enter the blood vessel, bind to endothelial cell heparan sulfate glycosaminoglycans (labeled "proteoglycan" in Figure 2-6), and are displayed at high concentrations on the endothelial surface.\[^{21}\] These chemokines act on the rolling leukocytes and activate the leukocytes. One of the consequences of activation is the conversion of VLA-4 and
LFA-1 integrins on the leukocytes to a high-affinity state. The combination of induced expression of integrin ligands on the endothelium and activation of integrins on the leukocytes results in firm integrin-mediated binding of the leukocytes to the endothelium at the site of infection. The leukocytes stop rolling, their cytoskeleton is reorganized, and they spread out on the endothelial surface.

The next step in the process is migration of the leukocytes through the endothelium, called transmigration or diapedesis. Chemokines act on the adherent leukocytes and stimulate the cells to migrate through interendothelial spaces toward the chemical concentration gradient, that is, toward the site of injury or infection. Certain homophilic adhesion molecules (i.e., adhesion molecules that bind to each other) present in the intercellular junction of endothelium are involved in the migration of leukocytes. One of these molecules is a member of the immunoglobulin superfamily called PECAM-1 (platelet endothelial cell adhesion molecule) or CD31. Leukocyte diapedesis, similar to increased vascular permeability, occurs predominantly in the venules (except in the lungs, where it also occurs in capillaries). After traversing the endothelium, leukocytes are transiently retarded in their journey by the continuous basement membrane of the venules, but eventually the cells pierce the basement membrane, probably by secreting collagenases. The net result of this process is that leukocytes rapidly accumulate where they are needed.

Once leukocytes enter the extravascular connective tissue, they are able to adhere to the extracellular matrix by virtue of $\beta_1$ integrins and CD44 binding to matrix proteins. Thus, the leukocytes are retained at the site where they are needed.

The most telling proof of the importance of adhesion molecules is the existence of genetic deficiencies in the leukocyte adhesion proteins, which result in impaired leukocyte adhesion and recurrent bacterial infections. In leukocyte adhesion deficiency type 1 (LAD1), patients have a defect in the biosynthesis of the $\beta_2$ chain shared by the LFA-1 and Mac-1 integrins. Leukocyte adhesion deficiency type 2 (LAD2) is caused by the absence of sialyl-Lewis X, the fucose-containing ligand for E-selectin, owing to a defect in a fucosyl transferase, the enzyme that attaches fucose moieties to protein backbones. In addition, antibodies to adhesion molecules abrogate leukocyte extravasation in experimental models of acute inflammation, and gene knockout mice deficient in these molecules show defects in leukocyte adhesion and extravasation.

The type of emigrating leukocyte varies with the age of the inflammatory response and with the type of stimulus. In most forms of acute inflammation, neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 hours, then are replaced by monocytes in 24 to 48 hours (Fig. 2-8). Several reasons account for this sequence—neutrophils are more numerous in the blood, they respond more rapidly to chemokines, and they may attach more firmly to the adhesion molecules that are rapidly induced on endothelial cells, such as P- and E-selectins. In addition, after entering tissues, neutrophils are short-lived; they undergo apoptosis and disappear after 24 to 48 hours, whereas monocytes survive longer. There are exceptions to this pattern of cellular exudation, however. In certain infections—for example, those produced by Pseudomonas organisms—neutrophils predominate over 2 to 4 days; in viral infections, lymphocytes may be the
first cells to arrive; in some hypersensitivity reactions, eosinophilic granulocytes may be the main cell type.

Figure 2-8  Schematic and histologic sequence of events following acute injury. The photomicrographs are representative of the early (neutrophilic) (left) and later (mononuclear) cellular infiltrates (right) of infarcted myocardium. The kinetics of edema and cellular infiltration are approximations. For sake of simplicity, edema is shown as an acute transient response, although secondary waves of delayed edema and neutrophil infiltration can also occur.

Chemotaxis

After extravasation, leukocytes emigrate in tissues toward the site of injury by a process called chemotaxis, defined most simply as locomotion oriented along a chemical gradient. All granulocytes, monocytes and, to a lesser extent, lymphocytes respond to chemotactic stimuli with varying rates of speed. Both exogenous and endogenous substances can act as chemoattractants. The most common exogenous agents are bacterial products. Some of these are peptides that possess an N-formyl-methionine terminal amino acid. Others are lipid in nature. Endogenous chemoattractants, which are detailed later, include several chemical mediators: (1) components of the complement system, particularly C5a; (2) products of the lipoxygenase pathway, mainly leukotriene B4 (LTB4); and (3) cytokines, particularly those of the chemokine family (e.g., IL-8).
How does the leukocyte sense the chemotactic agents, and how do these substances induce directed cell movement? Although not all the answers are known, several important steps and second messengers are recognized. All the chemotactic agents mentioned above bind to specific seven-transmembrane G-protein-coupled receptors (GPCRs) on the surface of leukocytes. Signals initiated from these receptors result in recruitment of G-proteins and activation of several effector molecules, including phospholipase C (PLC\(\gamma\)) and phosphoinositol-3 kinase (PI3K), as well as protein tyrosine kinases. (The general steps in these signal transduction pathways are described in Chapter 3.) PLC\(\gamma\) and PI3K act on membrane inositol phospholipids to generate lipid second messengers that increase cytosolic calcium and activate small GTPases of the Rac/Rho/cdc42 family as well as numerous kinases. The GTPases induce polymerization of actin, resulting in increased amounts of polymerized actin at the leading edge of the cell. The leukocyte moves by extending filopodia that pull the back of the cell in the direction of extension, much as an automobile with front-wheel drive is pulled by the wheels in front (Fig. 2-9). Actin reorganization may also occur at the trailing edge of the cell. Locomotion involves rapid assembly of actin monomers into linear polymers at the filopodium's leading edge, followed by cross-linking of filaments, and disassembly of such filaments away from the leading edge. A number of actin-regulating proteins, such as filamin, gelsolin, profilin, and calmodulin, interact with actin and myosin in the filopodium to produce contraction.

**Figure 2-9** Scanning electron micrograph of a moving leukocyte in culture showing a filopodium (upper left) and a trailing tail. (Courtesy of Dr. Morris J. Karnovsky, Harvard Medical School, Boston, MA.)
Microbes, products of necrotic cells, antigen-antibody complexes, and cytokines, including chemotactic factors, induce a number of responses in leukocytes that are part of the defensive functions of the leukocytes (neutrophils and monocytes/macrophages) and are referred to under the rubric of **leukocyte activation** (Fig. 2-10). Activation results from several signaling pathways that are triggered in leukocytes, resulting in increases in cytosolic Ca\(^{2+}\) and activation of enzymes such as protein kinase C and phospholipase A\(_2\). The functional responses that are induced on leukocyte activation include the following:

- **Production of arachidonic acid metabolites** from phospholipids, as a result of activation of phospholipase A\(_2\) by increased intracellular calcium and other signals.
- **Degranulation and secretion of lysosomal enzymes and activation of the oxidative burst** (discussed below under phagocytosis).
- **Secretion of cytokines**, which amplify and regulate inflammatory reactions. Activated macrophages are the chief source of the cytokines that are involved in inflammation, but mast cells and other leukocytes may contribute.
- **Modulation of leukocyte adhesion molecules**. As stated earlier, different cytokines cause increased endothelial expression of adhesion molecules and increased avidity of leukocyte integrins, allowing firm adhesion of activated neutrophils to endothelium.

**Figure 2-10** Leukocyte activation. Different classes of cell surface receptors of leukocytes recognize different stimuli. The receptors initiate responses that mediate the functions of the leukocytes. Only some receptors are depicted (see text for details).
Leukocytes express a number of surface receptors that are involved in their activation (see Fig. 2-10). These receptors include the following:

- **Toll-like receptors (TLRs)**, which are homologous to a Drosophila protein called Toll, function to activate leukocytes in response to different types and components of microbes. To date, 10 mammalian TLRs have been identified, and each appears to be required for responses to different classes of infectious pathogens (Chapter 6, Box 6-1). Different TLRs play essential roles in cellular responses to bacterial lipopolysaccharide (LPS, or endotoxin), other bacterial proteoglycans, and unmethylated CpG nucleotides, all of which are found only in bacteria, as well as double-stranded RNA, which is produced only by some viruses. These receptors function by receptor-associated kinases to stimulate the production of microbicidal substances and cytokines in the leukocytes.

- **Different seven-transmembrane G-protein-coupled receptors** recognize microbes and some mediators that are produced in response to infections and tissue injury. These receptors have a conserved structure with seven transmembrane α-helical domains; are found on neutrophils, macrophages, and most other types of leukocytes; and are specific for diverse ligands. Receptors of this class recognize short peptides containing N-formylmethionyl residues, as well as chemokines, chemotactic breakdown products of complement such as C5α, and lipid mediators of inflammation, including platelet-activating factor, prostaglandin E, and LTB4. Because all bacterial proteins and few mammalian proteins (only those synthesized within mitochondria) are initiated by N-formylmethionine, this receptor allows neutrophils to detect and respond to bacterial proteins. Binding of ligands, such as microbial products and chemokines, to the G-protein-coupled receptors induces migration of the cells from the blood through the endothelium and production of microbicidal substances by activation of the respiratory burst. In a resting cell, the receptor-associated G-proteins form a stable inactive complex containing guanosine diphosphate (GDP) bound to Gα subunits. Occupancy of the receptor by ligand results in an exchange of GTP for GDP. The GTP-bound form of the G-protein activates numerous cellular enzymes, including an isofrom of phosphatidylinositol-specific phospholipase C that functions to degrade inositol phospholipids and ultimately to increase intracellular CA2+ and activate protein kinase C. The G-proteins also stimulate cytoskeletal changes, resulting in increased cell motility.

- **Phagocytes express receptors for cytokines** that are produced during immune responses. One of the most important of these cytokines is IFN-γ, which is secreted by natural killer (NK) cells during innate immune responses and by antigen-activated T lymphocytes during adaptive immune responses. IFN-γ is the major macrophage-activating cytokine.

- **Receptors for opsonins** promote phagocytosis of microbes coated with various proteins and deliver signals that activate the phagocytes. The process of coating a particle, such as a microbe, to target it for phagocytosis is called opsonization, and substances that do this are opsonins. These substances include antibodies, complement proteins, and lectins. One of the most efficient systems for opsonizing particles is coating the particles with IgG antibodies, which are termed specific opsonins and are recognized by the high-affinity Fcγ receptor of phagocytes, called FcyRI (see Chapter 6). Components of the complement system, especially fragments of the complement protein C3, are also potent opsonins, because these
fragments bind to microbes and phagocytes express a receptor, called the type 1 complement receptor (CR1), that recognizes breakdown products of C3 (discussed later). These complement fragments are produced when complement is activated by either the classical (antibody-dependent) or the alternative (antibody-independent) pathway. Many bacteria can activate the alternative pathway and produce complement proteins that efficiently opsonize the bacteria in the absence of antibody molecules. A number of plasma proteins, including mannose-binding lectin (MBL), fibronectin, fibrinogen, and C-reactive protein, can coat microbes and are recognized by receptors on phagocytes. For example, a macrophage cell surface receptor called the C1q receptor binds microbes opsonized with plasma MBL, and integrins bind fibrinogen-coated particles.

Phagocytosis

Phagocytosis and the release of enzymes by neutrophils and macrophages are responsible for eliminating the injurious agents and thus constitute two of the major benefits derived from the accumulation of leukocytes at the inflammatory focus. Phagocytosis involves three distinct but interrelated steps (Fig. 2-11A): (1) recognition and attachment of the particle to be ingested by the leukocyte; (2) its engulfment, with subsequent formation of a phagocytic vacuole; and (3) killing or degradation of the ingested material.
Figure 2-11  A, Phagocytosis of a particle (e.g., bacterium) involves attachment and binding of Fc and C3b to receptors on the leukocyte membrane, engulfment, and fusion of lysosomes with phagocytic vacuoles, followed by destruction of ingested particles within the phagolysosomes. Note that during phagocytosis, granule contents may be released into extracellular tissues. B, Production of microbicidal reactive oxygen intermediates within phagocytic vesicles.

Recognition and Attachment.

Although neutrophils and macrophages can engulf bacteria or extraneous matter (e.g., latex beads) without attachment to specific receptors, typically the phagocytosis of microbes and dead cells is initiated by recognition of the particles by receptors expressed on the leukocyte surface. Mannose
receptors and scavenger receptors are two important receptors that function to bind and ingest microbes. The mannose receptor is a macrophage lectin that binds terminal mannose and fucose residues of glycoproteins and glycolipids. These sugars are typically part of molecules found on microbial cell walls, whereas mammalian glycoproteins and glycolipids contain terminal sialic acid or N-acetyl-galactosamine. Therefore, the macrophage mannose receptor recognizes microbes and not host cells. Scavenger receptors were originally defined as molecules that bind and mediate endocytosis of oxidized or acetylated low-density lipoprotein (LDL) particles that can no longer interact with the conventional LDL receptor. Macrophage scavenger receptors bind a variety of microbes in addition to modified LDL particles. Macrophage integrins, notably Mac-1 (CD11b/CD18), may also bind microbes for phagocytosis.

The efficiency of phagocytosis is greatly enhanced when microbes are opsonized by specific proteins (opsonins) for which the phagocytes express high-affinity receptors. As described above, the major opsonins are IgG antibodies, the C3b breakdown product of complement, and certain plasma lectins, notably MBL, all of which are recognized by specific receptors on leukocytes.

Engulfment.

Binding of a particle to phagocytic leukocyte receptors initiates the process of active phagocytosis of the particle. During engulfment, extensions of the cytoplasm (pseudopods) flow around the particle to be engulfed, eventually resulting in complete enclosure of the particle within a phagosome created by the plasma membrane of the cell. The limiting membrane of this phagocytic vacuole then fuses with the limiting membrane of a lysosomal granule, resulting in discharge of the granule's contents into the phagolysosome (see Fig. 2-11A). During this process, the neutrophil and the monocyte become progressively degranulated.

The process of phagocytosis is complex and involves the integration of many receptor-initiated signals with the coordinated orchestration of membrane remodeling and cytoskeletal changes. Phagocytosis is dependent on polymerization of actin filaments; it is, therefore, not surprising that the signals that trigger phagocytosis are many of the same that are involved in chemotaxis. (In contrast, fluid phase pinocytosis and receptor-mediated endocytosis of small particles involve internalization into clathrin-coated pits and vesicles and are not dependent on the actin cytoskeleton.)

Killing and Degradation.

The ultimate step in the elimination of infectious agents and necrotic cells is their killing and degradation within neutrophils and macrophages, which occur most efficiently after activation of the phagocytes. Microbial killing is accomplished largely by oxygen-dependent mechanisms (Fig. 2-11B). Phagocytosis stimulates a burst in oxygen consumption, glycogenolysis, increased glucose oxidation via the hexose-monophosphate shunt, and production of reactive oxygen intermediates (ROIs, also called reactive oxygen sepecies).

The generation of reactive oxygen intermediates is due to the rapid activation of an oxidase (NADPH oxidase), which oxidizes NADPH (reduced nicotinamide-adenine dinucleotide phosphate) and, in the process, reduces oxygen to superoxide anion (\( \text{O}_2^- \)). Superoxide is then converted
into hydrogen peroxide (H₂O₂), mostly by spontaneous dismutation. Hydrogen peroxide can also be further reduced to the highly reactive hydroxyl radical (OH). Most of the H₂O₂ is eventually broken down by catalase into H₂O and O₂, and some is destroyed by the action of glutathione oxidase. This process and its regulation were described in detail in Chapter 1.

NADPH oxidase is an enzyme complex consisting of at least seven proteins. In resting neutrophils, different NADPH oxidase protein components are located in the plasma membrane and the cytoplasm. In response to activating stimuli, the cytosolic protein components translocate to the plasma membrane or phagosomal membrane, where they assemble and form the functional enzyme complex (see Fig. 2-11B). Thus, the reactive oxygen intermediates are produced within the lysosome where the ingested substances are segregated, and the cell’s own organelles are protected from the harmful effects of the ROIs. A similar enzyme system generates reactive nitrogen intermediates, notably nitric oxide, which also helps to kill microbes.

The H₂O₂ generated by the NADPH oxidase system is generally not able to efficiently kill microbes by itself. However, the azurophilic granules of neutrophils contain the enzyme myeloperoxidase (MPO), which, in the presence of a halide such as Cl⁻, converts H₂O₂ to hypochlorite (HOCl). The latter is a potent antimicrobial agent that destroys microbes by halogenation (in which the halide is bound covalently to cellular constituents) or by oxidation of proteins and lipids (lipid peroxidation). The H₂O₂-MPO-halide system is the most efficient bactericidal system in neutrophils. MPO-deficient leukocytes are capable of killing bacteria (albeit more slowly than normal cells), by virtue of the formation of superoxide, hydroxyl radicals, and singlet-oxygen.

Bacterial killing can also occur by oxygen-independent mechanisms, through the action of substances in leukocyte granules. These include bactericidal permeability increasing protein (BPI), a highly cationic granule-associated protein that causes phospholipase activation, phospholipid degradation, and increased permeability in the outer membrane of the microorganisms; lysozyme, which hydrolyzes the muramic acid-N-acetyl-glucosamine bond, found in the glycopeptide coat of all bacteria; lactoferrin, an iron-binding protein present in specific granules; major basic protein, a cationic protein of eosinophils, which has limited bactericidal activity but is cytotoxic to many parasites; and defensins, cationic arginine-rich granule peptides that are cytotoxic to microbes (and certain mammalian cells). In addition, neutrophil granules contain many enzymes, such as elastase, that also contribute to microbial killing (discussed later in the chapter).

After killing, acid hydrolases, which are normally stored in lysosomes, degrade the microbes within phagolysosomes. The pH of the phagolysosome drops to between 4 and 5 after phagocytosis, this being the optimal pH for the action of these enzymes.

Release of Leukocyte Products and Leukocyte-Induced Tissue Injury

During activation and phagocytosis, leukocytes release microbicidal and other products not only within the phagolysosome but also into the extracellular space. The most important of these substances in neutrophils and macrophages are lysosomal enzymes, present in the granules; reactive oxygen intermediates; and products of arachidonic acid metabolism, including prostaglandins and leukotrienes. These products are capable of causing endothelial injury and
tissue damage and may thus amplify the effects of the initial injurious agent. Products of monocytes/macrophages and other leukocyte types have additional potentially harmful products, which are described in the discussion of chronic inflammation. Thus, if persistent and unchecked, the leukocyte infiltrate itself becomes the offender, and leukocyte-dependent tissue injury underlies many acute and chronic human diseases (Table 2-2). This fact becomes evident in the discussion of specific disorders throughout this book.

Table 2-2 -- Clinical Examples of Leukocyte-Induced Injury

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>Arthritis</td>
</tr>
<tr>
<td>Acute transplant rejection</td>
<td>Asthma</td>
</tr>
<tr>
<td>Asthma</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>Chronic lung disease</td>
</tr>
<tr>
<td>Reperfusion injury</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>Septic shock</td>
<td></td>
</tr>
<tr>
<td>Vasculitis</td>
<td></td>
</tr>
</tbody>
</table>

Regulated secretion of lysosomal proteins is a peculiarity of leukocytes and other hematopoietic cells. (Recall that in most secretory cells, the proteins that are secreted are not stored within lysosomes.) The contents of lysosomal granules are secreted by leukocytes into the extracellular milieu by diverse mechanisms. Release may occur if the phagocytic vacuole remains transiently open to the outside before complete closure of the phagolysosome (regurgitation during feeding). If cells are exposed to potentially ingestible materials, such as immune complexes deposited on immovable flat surfaces (e.g., glomerular basement membrane), attachment of leukocytes to the immune complexes triggers leukocyte activation, but the fixed immune complexes cannot be phagocytosed, and lysosomal enzymes are released into the medium (frustrated phagocytosis). Cytotoxic release occurs after phagocytosis of potentially membranolytic substances, such as urate crystals, which damage the membrane of the phagolysosome. In addition, there is some evidence that proteins in certain granules, particularly the specific (secondary) granules of neutrophils, may be directly secreted by exocytosis.

After phagocytosis, neutrophils rapidly undergo apoptotic cell death and are ingested by macrophages.

Defects in Leukocyte Function

From the preceding discussion, it is obvious that leukocytes play a central role in host defense. Not surprisingly, therefore, defects in leukocyte function, both genetic and acquired, lead to increased vulnerability to infections (Table 2-3). Impairments of virtually every phase of leukocyte function—from adherence to vascular endothelium to microbicidal activity—have been identified,
and the existence of clinical genetic deficiencies in each of the critical steps in the process has been described. These include the following:

\* **Defects in leukocyte adhesion.** We previously mentioned the genetic deficiencies in leukocyte adhesion molecules (LAD types 1 and 2). LAD 1 is characterized by recurrent bacterial infections and impaired wound healing. LAD 2 is clinically milder than LAD 1 but is also characterized by recurrent bacterial infections.

\* **Defects in phagolysosome function.** One such disorder is Chédiak-Higashi syndrome, an autosomal recessive condition characterized by neutropenia (decreased numbers of neutrophils), defective degranulation, and delayed microbial killing. The neutrophils (and other leukocytes) have giant granules, which can be readily seen in peripheral blood smears and which are thought to result from aberrant organelle fusion. In this syndrome, there is reduced transfer of lysosomal enzymes to phagocytic vacuoles in phagocytes (causing susceptibility to infections) and abnormalities in melanocytes (leading to albinism), cells of the nervous system (associated with nerve defects), and platelets (generating bleeding disorders). The gene associated with this disorder encodes a large cytosolic protein that is apparently involved in vesicular traffic but whose precise function is not yet known. The secretion of granule proteins by cytotoxic T cells is also affected, accounting for part of the immunodeficiency seen in the disorder.

\* **Defects in microbicidal activity.** The importance of oxygen-dependent bactericidal mechanisms is shown by the existence of a group of congenital disorders with defects in bacterial killing called chronic granulomatous disease, which render patients susceptible to recurrent bacterial infection. Chronic granulomatous disease results from inherited defects in the genes encoding several components of NADPH oxidase, which generates superoxide. The most common variants are an X-linked defect in one of the plasma membrane-bound components (gp91phox) and autosomal recessive defects in the genes encoding two of the cytoplasmic components (p47phox and p67phox).

\* Clinically, the most frequent cause of leukocyte defects is bone marrow suppression, leading to reduced production of leukocytes. This is seen following therapies for cancer (radiation and chemotherapy) and when the marrow space is compromised by tumor metastases to bone.

**Table 2-3 -- Defects in Leukocyte Functions**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td></td>
</tr>
<tr>
<td>Leukocyte adhesion deficiency 1</td>
<td>β chain of CD11/CD18 integrins</td>
</tr>
<tr>
<td>Leukocyte adhesion deficiency 2</td>
<td>Fucosyl transferase required for synthesis of sialylated oligosaccharide (receptor for selectin)</td>
</tr>
<tr>
<td>Chronic granulomatous disease</td>
<td>Decreased oxidative burst</td>
</tr>
<tr>
<td>X-linked</td>
<td>NADPH oxidase (membrane component)</td>
</tr>
<tr>
<td>Autosomal recessive</td>
<td>NADPH oxidase (cytoplasmic components)</td>
</tr>
<tr>
<td>Disease</td>
<td>Defect</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Myeloperoxidase deficiency</td>
<td>Absent MPO-H₂O₂ system</td>
</tr>
<tr>
<td>Chédiak-Higashi syndrome</td>
<td>Protein involved in organelle membrane docking and fusion</td>
</tr>
<tr>
<td><strong>Acquired</strong></td>
<td></td>
</tr>
<tr>
<td>Thermal injury, diabetes, malignancy, sepsis,</td>
<td>Chemotaxis</td>
</tr>
<tr>
<td>immunodeficiencies</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis, diabetes mellitus</td>
<td>Adhesion</td>
</tr>
<tr>
<td>Leukemia, anemia, sepsis, diabetes, neonates,</td>
<td>Phagocytosis and microbicidal activity</td>
</tr>
<tr>
<td>malnutrition</td>
<td></td>
</tr>
</tbody>
</table>


Although we have emphasized the role of leukocytes recruited from the circulation in the acute inflammatory response, cells resident in tissues also serve important functions in initiating acute inflammation. The two most important of these cell types are mast cells and tissue macrophages. Mast cells react to physical trauma, breakdown products of complement, microbial products, and neuropeptides. The cells release histamine, leukotrienes, enzymes, and many cytokines (including TNF, IL-1, and chemokines), all of which contribute to inflammation. The functions of mast cells are discussed in more detail in Chapter 6. Macrophages recognize microbial products and secrete most of the cytokines important in acute inflammation. These cells are stationed in tissues to rapidly recognize potentially injurious stimuli and initiate the host defense reaction.

**TERMINATION OF THE ACUTE INFLAMMATORY RESPONSE**

It is predictable that such a powerful system of host defense, with its inherent capacity to cause tissue damage, needs tight controls to minimize the damage. In part, inflammation declines simply because the mediators of inflammation have short half-lives, are degraded after their release, and are produced in quick bursts, only as long as the stimulus persists. In addition as inflammation develops, the process also triggers a variety of stop signals that serve to actively terminate the reaction. These active mechanisms include a switch in the production of pro-inflammatory leukotrienes to anti-inflammatory lipoxins from arachidonic acid (described below); the liberation of an anti-inflammatory cytokine, transforming growth factor-β (TGF-β), from macrophages and other cells; and neural impulses (cholinergic discharge) that inhibit the production of TNF in macrophages. There are, in addition, many other controls whose existence is suspected from the phenotypes of mice in which genes encoding putative regulatory molecules have been knocked out—these mice develop uncontrolled inflammation, but precisely how the regulation works normally is not yet defined. Not surprisingly, there is great interest in defining the molecular basis of the brakes on inflammation, since this knowledge could be used to design powerful anti-inflammatory drugs.