11

2 CHAPTER 2 How Inflammation Is Triggered

2.1 KEY POINTS

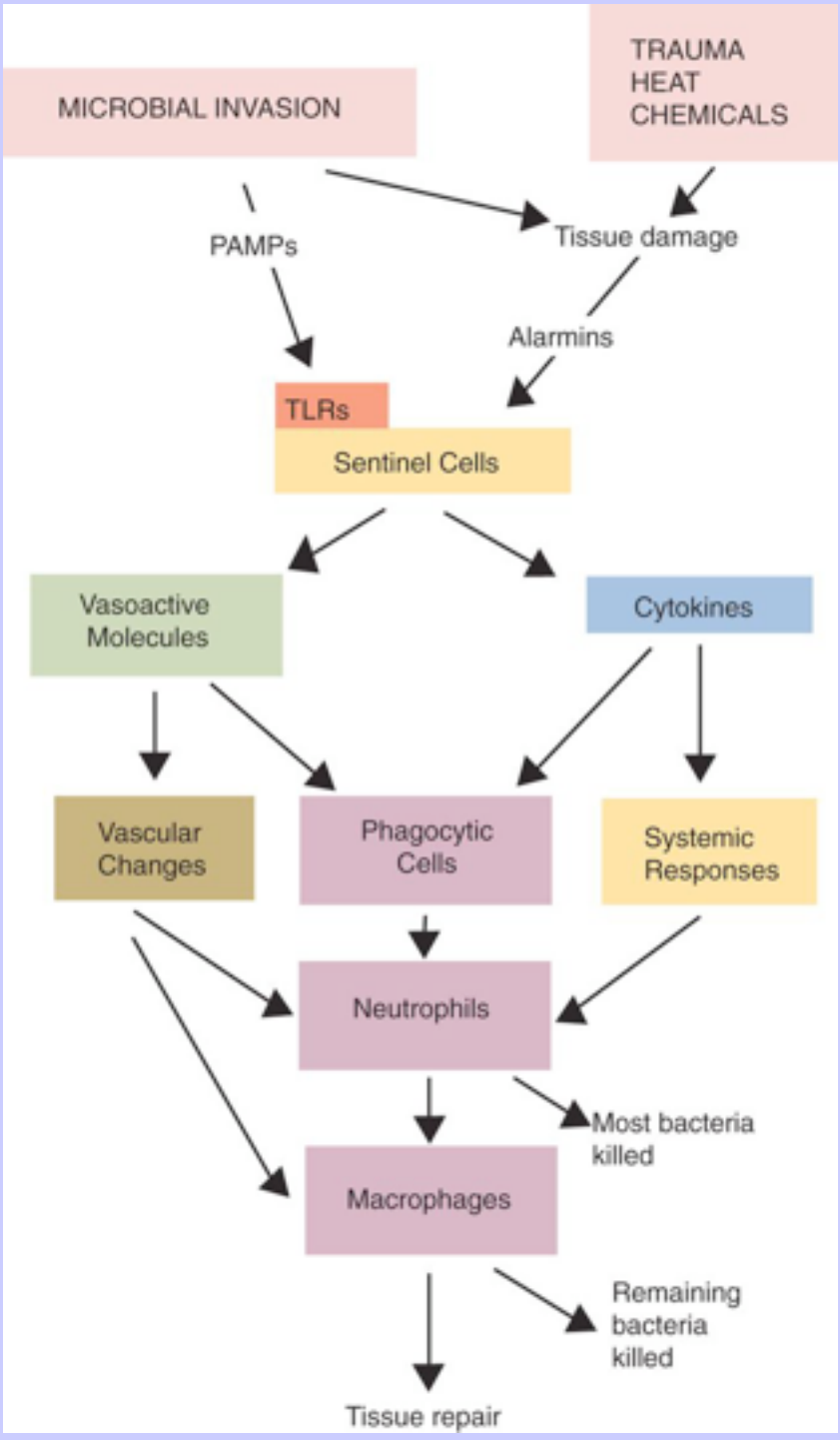
- The body recognizes invading microorganisms by the common molecules expressed on their surface, or by their characteristic nucleic acids. These are called pathogen-associated molecular patterns (PAMPs). It also recognizes the appearance of molecules released from damaged tissues. These are called alarmins.
- PAMPs are recognized by toll-like receptors (TLRs) on cell surfaces and by other receptors located within cells.
- Pattern-recognition receptors are found on many cell types. The most important cells are macrophages, dendritic cells (DCs), and mast cells, since these act as sentinels.
- Signals from TLRs cause the sentinel cells to be activated and to secrete many different molecules. Some of these molecules are cytokines that “turn on” the inflammatory process.
- The major proinflammatory cytokines are tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) as well as many different chemokines.
- Some of these molecules trigger local increases in blood flow and increased vascular permeability.

Infectious agents such as bacteria or viruses can grow very rapidly. A single bacterium with a doubling time of 50 minutes can produce about 500 million offspring within 24 hours. Thus if such a microorganism invades the body, it must be recognized and destroyed before it overwhelms the body. Time is of the essence, and delay can be fatal. The body must therefore employ fast-reacting preexisting immune mechanisms as its first line of defense against these invaders. The most important of these innate mechanisms is the process of acute inflammation.

Inflammation is vital because it ensures that defensive cells and molecules are concentrated rapidly at sites of microbial invasion and tissue damage. Inflammation involves the activation and directed migration of many different cells, especially neutrophils and macrophages, from the bloodstream to sites of invasion. Cells such as neutrophils are normally restricted to the bloodstream. They must migrate into infected tissues in order to destroy invaders. Likewise, many protective molecules, such as antibodies and complement components, are normally found only in blood.

11

FIGURE 2-1 An overview of the essential features of acute inflammation, an innate mechanism for focusing cells and other defensive mechanisms. It is triggered by microbial invasion and tissue damage.



These large molecules enter the tissues only during inflammation. Inflammation thus provides a mechanism by which defenses are focused in a localized region (Figure 2-1). Inflammation permits cells and molecules to attack and destroy invaders. Later, when the invader is eliminated, the repair of damaged tissues can begin.

2.2 HOW INVADERS ARE RECOGNIZED

Inflammation is triggered when the body senses that it is under attack. This involves recognizing warning signals generated either by invading microorganisms or by dead and dying cells. There are two major groups of such warning signals. One group consists of molecules released from dead or dying cells. These are called alarmins. The other group consists of molecules or molecular patterns associated with microbial invaders. Collectively, these are called pathogen-associated molecular patterns (PAMPs). Together, the internally generated alarmins and the externally generated PAMPs form a family of damage-associated molecular patterns that can be recognized by cells dedicated to the body's defenses.

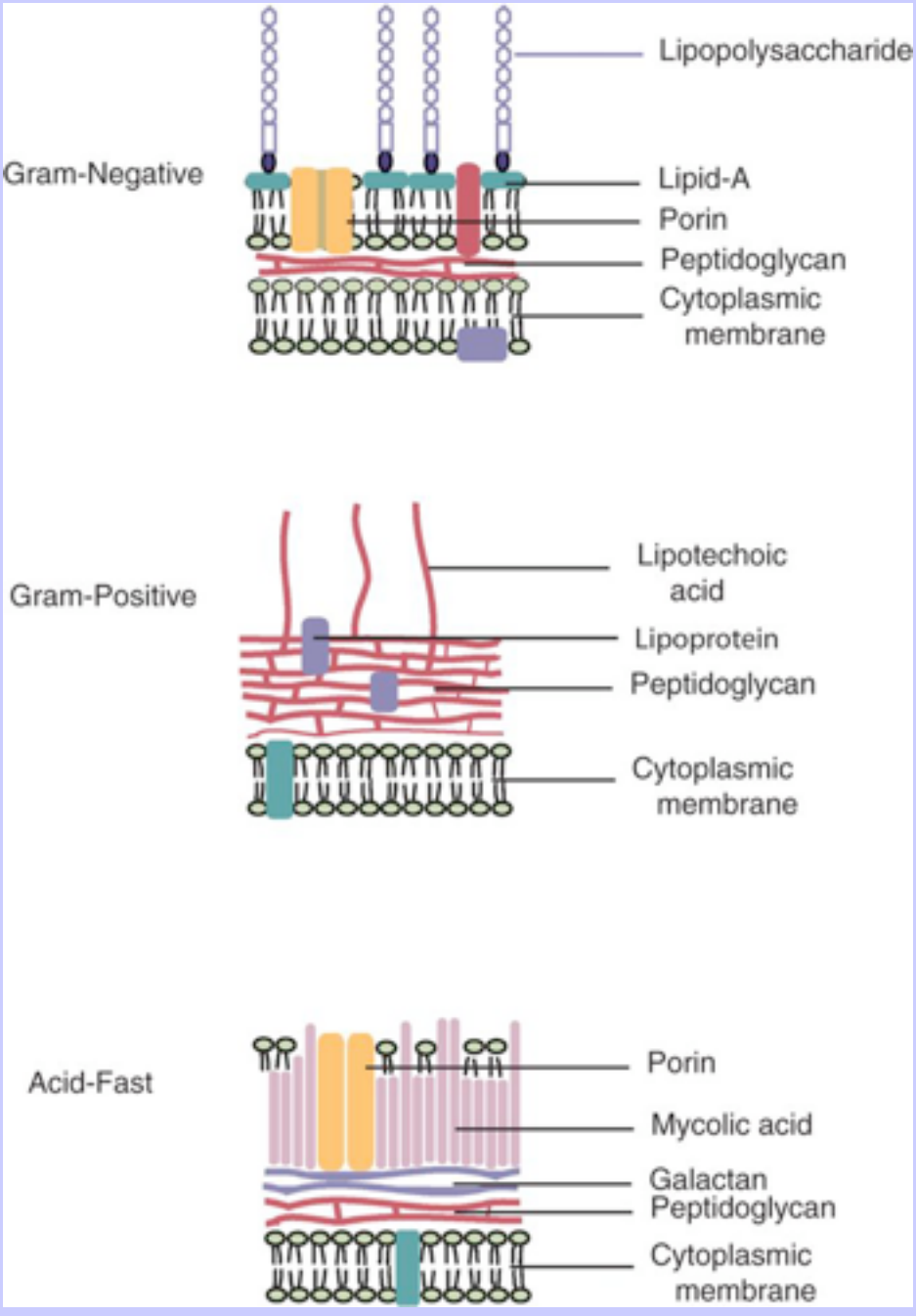
2.2.1 Pathogen-Associated Molecular Patterns

The presence of invading microbes is detected by “sentinel cells” such as macrophages, dendritic cells (DCs), and mast cells. These cells have receptors that can bind PAMPs expressed by bacteria, fungi, and viruses. Microbes not only grow fast, but also are highly diverse and can mutate and change their molecular structures much faster than any infected animal can respond. For this reason, sentinel cell receptors are not designed to recognize all possible microbial molecules. Rather, these cells use receptors that detect highly conserved molecules that are found in many different microorganisms. For example, most invasive bacteria are covered by a cell wall largely composed of complex carbohydrates. The walls of Gram-positive bacteria are largely composed of peptidoglycans (chains of alternating N-acetylglucosamine and N-acetylmuramic acid cross-linked by short peptide side chains) (Figure 2-2). Gram-positive bacterial cell walls also contain lipoteichoic acids. The cell walls of Gram-negative bacteria consist of peptidoglycans covered by a layer of lipopolysaccharide (LPS). Acid-fast bacteria are covered in glycolipids. Yeasts are also covered by a mannan-rich carbohydrate wall. None of these molecules is found in normal animal tissues. On the other hand, they are essential for microbial survival and are commonly shared by entire classes of pathogens. These PAMPs are therefore recognized by a set of “pattern-recognition receptors.” Many different pattern-recognition receptors have been identified, including receptors located on the cell surface and some located within the cytoplasm of the sentinel cells. Binding of PAMPs to these receptors activates intracellular signaling pathways and causes the sentinel cells to secrete molecules that trigger inflammation and other innate immune responses.

2.2.2 Toll-like Receptors

The most important of the pattern-recognition receptors are called toll-like receptors (TLRs). Some TLRs are located on cell surfaces, where they are well placed to encounter extracellular invaders. However, because

FIGURE 2-2 The major structural features of the cell walls of Gram-negative, Gram-positive, and acid-fast bacteria. These conserved structural molecules serve as pathogen-associated molecular patterns and can bind to pattern-recognition receptors such as the toll-like receptors.



viruses grow within cells, they must be detected by intracellular TLRs.

Table 2-1 PAMPs Recognized by the Mammalian Toll-like Receptors

TLR	Natural Ligands
TLR1	Diacylated lipoproteins
TLR2	Peptidoglycan, bacterial lipoproteins, zymosan, some LPS, spirochetes, mycobacteria, lipoteichoic acid, heat-shock protein, necrotic cells
TLR3	Double-stranded viral RNA
TLR4	LPS, lipoteichoic acid, viral protein, heat-shock protein, fibrinogen, saturated fatty acids, β-defensins, heparan sulfate
TLR5	Flagellin and flagellated bacteria
TLR6	Necrotic cells, diacylated lipoprotein, peptidoglycan (with TLR2)
TLR7	Single and double-stranded viral RNA
TLR8	Single-stranded viral RNA
TLR9	Unmethylated CpG bacterial DNA
TLR10	A pseudogene

TLRs are expressed by many different cell types. Most importantly, they are expressed on sentinel cells located on or near the surface of the body. These include macrophages, mast cells, and DCs, as well as eosinophils and the epithelial cells that line the respiratory and intestinal tracts. They owe their name to a closely related receptor called “Toll,” which was first identified in the fruit fly *(Drosophila)*.

TLRs are single-chain, membrane glycoproteins. There are at least 14 different TLRs, numbered accordingly, and each serves as a receptor for one or more specific microbial molecules (Table 2-1). TLRs may either be expressed on cell surfaces (TLR2, 4, and 5) or within cells on endosomal membranes (TLR3, 7, and 9).

The cell-surface TLRs mainly recognize microbial proteins, lipoproteins, and LPS. The intracellular TLRs recognize viral nucleic acids. For example, TLR4 on the cell surface binds LPS from the surface of Gram-negative bacteria. TLR2, on the other hand, recognizes peptidoglycans, lipoproteins, and a glycolipid called lipoarabinomannan from *Mycobacterium tuberculosis*. TLR5 binds flagellin, the major protein of bacterial flagella. TLR9 is a cytoplasmic receptor for bacterial deoxyribonucleic acid (DNA). Bacteria must therefore be disrupted if this DNA is to be recognized. Both TLR3 and TLR7 bind viral double-stranded ribonucleic acid (RNA), whereas TLR 7 and TLR 8 are required for the recognition of viral single-stranded RNA. TLRs may also cooperate to bind PAMPs. For example, TLR2 can associate with TLR6, and the dual receptor complex can then recognize bacterial lipopeptides. Likewise, TLR1 associates with TLR2 to recognize mycobacterial lipoprotein. Given the number of possible TLR combinations, it is believed that the presently known TLRs can collectively recognize almost all infectious agents. TLR11 is somewhat different from the other TLRs. It is restricted to DCs, macrophages, and epithelial cells in the urinary tract, where it binds to bacteria and some PAMPs from protozoan parasites.

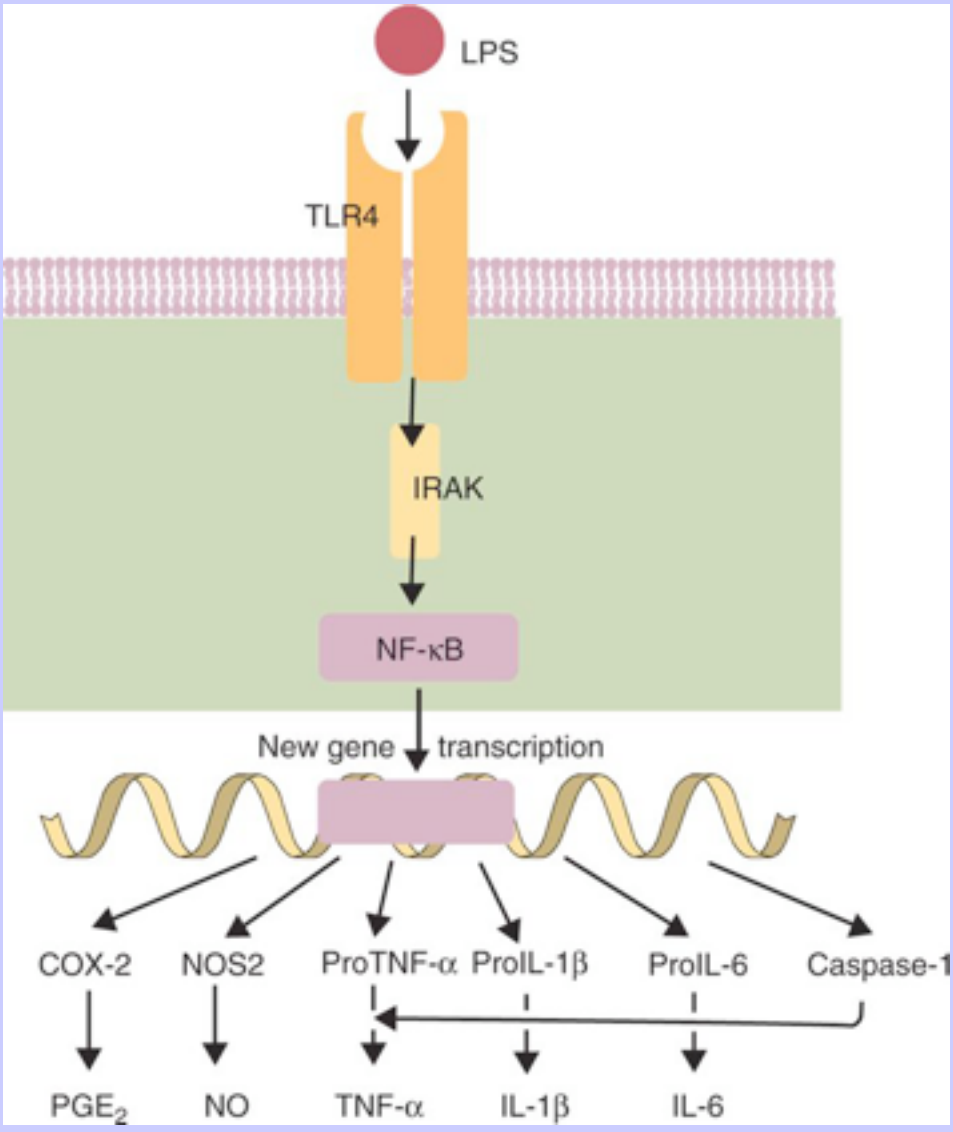
Once a cell-surface TLR binds a microbial PAMP (its ligand), a signal is passed to the cell. This results in an increase in a transcription factor called nuclear factor kappa-B (NF-κB) (Figure 2-3). NF-κB in turn activates the

genes that encode the cytokines, interleu-kin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). (For additional details see Chapter 6.) Cytokines are proteins that regulate the activities

13

14

FIGURE 2-3 Binding of a pathogen-associated molecular pattern such as lipopolysaccharide to a toll-like receptor (TLR) leads to generation of a transcription factor called nuclear factor kappa-B (NF-κB). NF-κB turns on the genes for three major cytokines, interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α). It also turns on the genes for nitric oxide synthase 2 (NOS2) and cyclooxygenase-2 (COX-2). These two enzymes generate nitric oxide and prostaglandins and leukotrienes, respectively.



of cells involved in the defense of the body. The cytokines are first made as pro-molecules that have to be activated by an enzyme called caspase-1. The production of caspase-1 is stimulated by a protein complex called an inflammasome that forms when microbial molecules bind to TLRs.

Table 2-2 Other Mammalian Pattern-Recognition Receptors

Receptor	Natural Ligands
Mannose-fucose receptor	Terminal mannose/fucose on microbial glycoproteins and glycolipids
CD14	Bacterial lipopolysaccharides
NOD1	Bacterial peptidoglycans
NOD2	Muramyl dipeptide
Peptidoglycan-recognition proteins	Bacterial peptidoglycans
RIG-like receptors	Viral RNA
CD1	Bacterial glycolipids
CD36	Bacterial lipoproteins
CD48	Fimbrial proteins

Caspases are a family of proteolytic enzymes, the cysteinyl-aspartate specific proteinases, that play key roles in the initiation of inflammation. Some of these caspases, such as caspase-1, 4, 5, and 12, are activated by signals generated by TLRs. Caspase-1 is the most important in this respect. It acts on the inactive precursors of IL-1, IL-6, and TNF-α to produce the active cytokines. These cytokines trigger the next phase of the inflammatory response.

Different TLRs trigger the production of different cytokine mixtures, and different PAMPs trigger distinctly different responses even within one cell type. For example, TLRs that recognize bacterial molecules tend to trigger the production of cytokines optimized to combat bacteria; those that recognize viral molecules will produce antiviral cytokines, and so forth.

TLRs not only trigger the innate immune defenses such as inflammation but also begin the process of “turning on” the acquired immune system. For example, stimulation of TLR4 makes macrophages and their close relatives, the DCs, produce cytokines that are potent stimulators of immune cells (see Chapter 8).

TLRs are expressed on hematopoietic stem cells—the bone marrow cells that produce leukocytes. Bacterial LPS binding to TLR4 stimulate the differentiation of these stem cells into leukocyte progenitors and cause the bone marrow to increase leukocyte production. An increase in leukocyte numbers in the blood (the white cell count) is a consistent feature of infectious diseases. This pathway also stimulates the development of DCs from lymphoid progenitors and so activates and replenishes the innate immune system during infections.

2.2.3 NOD-like Receptors

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a family of pattern-recognition receptors found inside cells. Unlike TLRs, which mainly detect extracellular microbes, NLRs can detect pathogens within the cytosol and when activated induce host defense signaling pathways (Table 2-2). Although TLRs and NLRs differ in their location and function, they share similar structures for microbial sensing and

cooperate to initiate host responses to pathogens. NOD1 recognizes bacterial peptidoglycans. NOD2 recognizes muramyl dipeptide and serves as a general sensor of intracellular bacteria. Both NOD proteins act to generate NF-κB.

14

15

2.2.4 Peptidoglycan-Recognition Proteins

Peptidoglycans are polymers of alternating N-acetyl glucosamine and N-acetyl muraminic acid found on both Gram-positive and Gram-negative bacteria. Peptidoglycan-recognition proteins (PGRPs) bind these microbial peptidoglycans and induce the production of antimicrobial peptides such as defensins. Although first identified in arthropods, they have since been found in humans, mice, cattle, and pigs. In pigs they are expressed constitutively in the skin, bone marrow, intestine, liver, kidney, and spleen. Their expression in intestinal tissues is increased by Salmonella infection. One member of this family, bovine PGRP-S, can kill microorganisms in which the peptidoglycan is either buried (Gram-negative bacteria) or absent *(Cryptococcus),* thus raising questions about its precise ligand. PGRP-S also binds bacterial LPS and lipotechoic acids. It is localized on the large granules of naïve neutrophils, which release PGRP-S when exposed to bacteria. Thus PGRP-S probably plays a significant role in the resistance of cattle to bacterial infections.

2.2.5 RIG-like Receptors

Retinoic acid inducible gene (RIG)-like receptors (RLRs) are pattern-recognition receptors expressed in the cytosol of cells, where they bind viral RNA. Viral RNA is different in several respects from mammalian RNA and so can be detected by these molecules. On interacting with viral RNA, RLRs initiate a cellular antiviral response and the production of antiviral cytokines called interferons.

2.2.6 Other Pattern-Recognition Receptors

The sentinel cells—macrophages, mast cells, and DCs—have many other receptors that can recognize microbial molecules. These include mannan receptors that bind microbial carbohydrates; scavenger receptors such as CD36 that can bind bacterial lipoproteins, and CD1, which binds microbial glycolipids.

2.2.7 Bacterial DNA

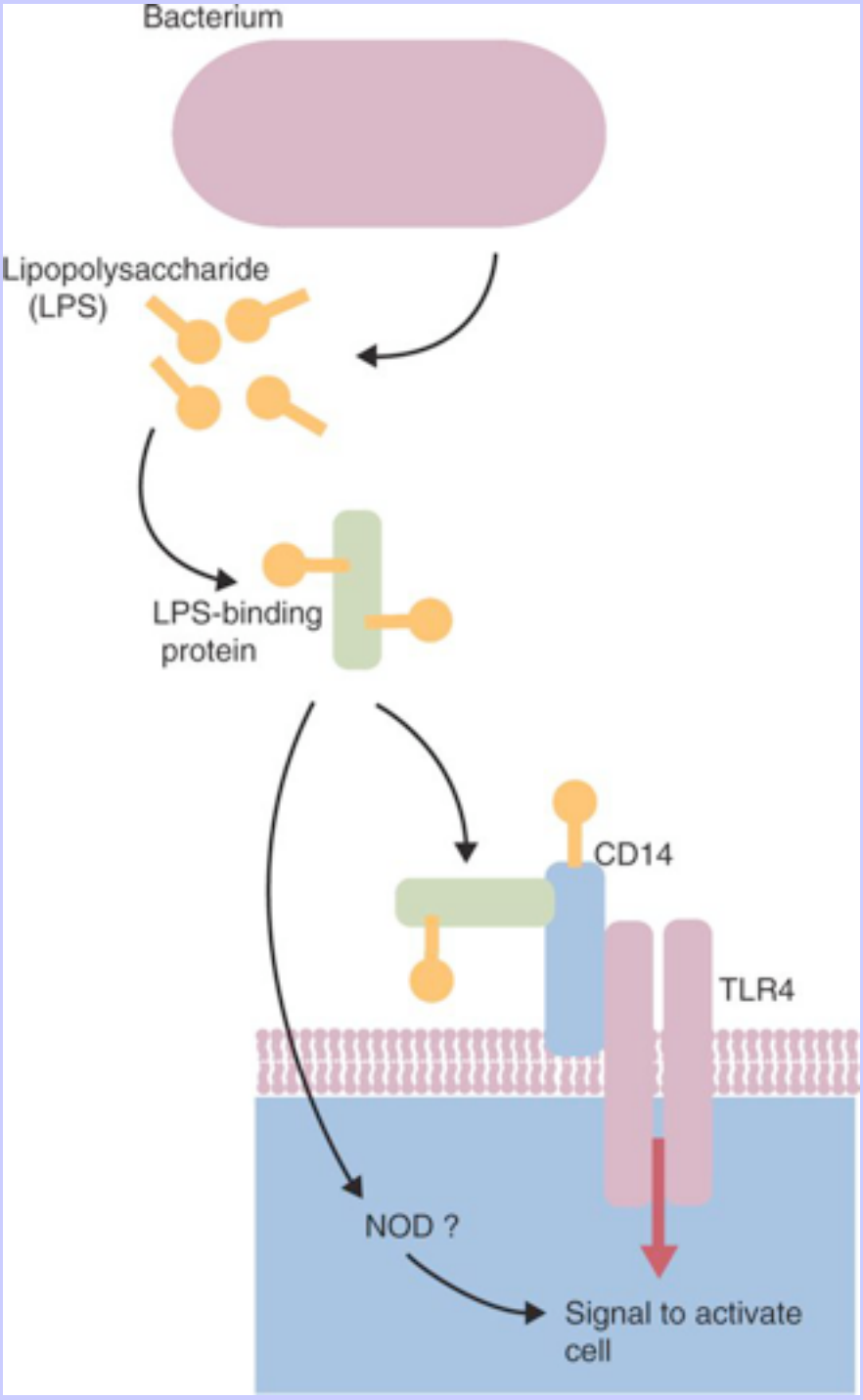
Bacterial DNA stimulates innate immunity. It differs from eukaryotic DNA in that it contains a large proportion of the dinucleotide cytosine-guanosine (CpG). In addition, while the cytosine in eukaryotic DNA is normally methylated, this is not the case in bacterial DNA. Thus unmethylated CpG dinucleotides are sufficiently different that they can bind TLR9 and so trigger innate immune responses. Bacterial DNA also contains deoxyguanosine (dG) nucleotides. These dG nucleotides form structures other than the usual double helix. One such structure is called quadriplex DNA. This binds to TLR9 and stimulates production of the cytokines IL-12, TNF-α, and IL-6.

2.2.8 Bacterial Lipopolysaccharides

Bacterial LPS are potent inducers of innate immunity. They are released by invading Gram-negative bacteria. They do not act directly on cells but first bind to LPS-binding protein (LBP) in serum (Figure 2-4). LBP immediately transfers LPS molecules to a protein called CD14 located on the surface of macrophages (Box 2-1). CD14 cannot penetrate cell membranes and so is unable to signal to cells directly. CD14 therefore binds to TLR4 on the cell surface. Binding of LPS to the CD14/TLR4 complex activates macrophages and triggers cytokine

production. The LPS subsequently dissociates from CD14 and binds to lipoproteins, where its toxic activities are lost. CD14 also binds many other microbial molecules: lipoarabinomannans from mycobacteria, manuronic acid polymers from *Pseudomonas,* and peptidoglycans from *Staphylococcus aureus*.

FIGURE 2-4 The processing and fate of bacterial lipo-polysaccharide.



2.2.8.1

Box 2-1 The CD System

When advances in immunology made it possible to make highly specific antibodies against individual cell surface proteins (see Chapter 13), it was soon shown that mammalian cells possessed hundreds of different surface proteins. Initially, each protein was given a specific name and often an acronym as well. It soon became clear, however, that such a system was unworkable. In an attempt to classify these proteins, a system has been established that assigns each protein to a numbered cluster of differentiation (CD). In many cases, a defined CD denotes a protein of specific function. For example, the protein CD14 binds bacterial lipopolysaccharide. As of May 2006, numbers up to CD350 have been assigned. Unfortunately, CD numbers provide no clue as to a molecule's function. As a result, in practice immunologists tend to use a mixed system using both a CD number and an abbreviation that denotes the function of a molecule. For example, CD32 is also called FcgR1. A list of selected CD molecules can be found in Appendix 1.

2.2.9

The Complement System

Perhaps the most important of the innate protective systems that can destroy invading microbes is the complement system. This system consists of a large number of proteins found in the bloodstream. When exposed to invading bacteria, the complement system is activated through reactions involving several distinct pathways. For example, it can be triggered simply by exposure of complement proteins to microbial cell walls. This method of activation is called the alter-native complement pathway. Another complement pathway is activated when mannose-binding lectin encounters microbial cell walls. Once activated by either pathway, activated complement components can either kill microbes directly or prepare them for capture by phagocytic (eating) cells. The complement system is described in detail in Chapter 5.

2.3

ALARMINS

The innate immune system must recognize not only PAMPs derived from invading microorganisms but also molecules released by damaged tissues. These molecules, collectively called alarmins, may be released when cells die. Alternatively, they may be secreted by stimulated sentinel cells. Alarmins are multifunctional and many have potent antimicrobial properties. They may recruit and activate cells of the innate immune system and indirectly promote acquired immune responses. Many different molecules can act as alarmins. They include defensins, cathelicidins, eosinophil-derived neurotoxin, and high mobility group box protein-1 (HMGB1). Other molecules that may be classified as alarmins include some chemokines, cytokines such as interleukin-1a (IL-1α), galectin-1, and S100 proteins (a family of calcium-binding proteins involved in cell growth and tissue injury). All are released in response to tissue damage and play key roles in innate immunity and tissue repair.

An example of an alarmin is heparan sulfate generated by broken cells. This molecule is normally restricted to cell membranes and the extracellular matrix but is shed into tissue fluids following injury. Heparan sulfate binds to TLR4 and so activates sentinel cells. Fibrinogen, a clotting protein, also stimulates macrophages through TLR4. Other alarmins include heat-shock proteins synthesized by cells under stress. These proteins bind TLR2 and TLR4.

2.3.1

HMGB1

HMGB1 was first described as a histone, a protein that binds DNA and ensures the correct folding of DNA molecules within the nucleus. It is found in all vertebrate cells and is highly conserved between species. However, HMGB1 has a second function. It is a cytokine with the ability to trigger inflammation. Thus HMGB1 is secreted

by macrophages activated by LPS or proinflammatory cytokines such as interferon-g. HMGB1 also leaks from necrotic or damaged and dying cells. Apoptotic cells, in contrast, do not release HMGB1 since these cells retain their nuclear integrity. HMGB1 binds to TLR2 and TLR4 and so stimulates cytokine production. HMGB1 sustains and prolongs inflammation since it induces the secretion of proinflammatory cytokines from macrophages, monocytes, neutrophils, and endothelial cells. Administration of HMGB1 to normal animals causes fever, weight loss, anorexia, acute lung injury, arthritis, and death. It plays a role in tissue repair since it stimulates the growth of new blood vessels. HMGB1 has potent antimicrobial activity. DCs can also secrete HMGB1 and this in turn promotes the proliferation and Th1 polarization of interacting T cells (see Chapter 12).

2.4 SENTINEL CELLS

The major sentinel cells—macrophages, DCs, and mast cells—are scattered throughout the body but are found in highest numbers just below body surfaces at locations where invading microorganisms are likely to be encountered. All can detect and then respond rapidly to PAMPs and alarmins.

2.4.1 Macrophages

Macrophages not only act as sentinel cells by detecting invading microorganisms, they can also kill invaders and play an essential role in triggering acquired immunity. When stimulated, they secrete cytokines that promote both innate and acquired immune responses; they control inflammation; and they contribute directly to the repair of damaged tissues by removing dead, dying, and damaged cells and so assist the healing process. Their name is derived from the fact that they are “large-eating” cells (Greek *macro, phage*).

Immature macrophages circulate in the blood, where they are called monocytes. When monocytes mature they migrate into tissues and become macrophages. Mature macrophages found in connective tissue are called histiocytes; those lining the sinusoids of the liver are called Kupffer cells; those in the brain are microglia. The macrophages found in the alveoli of the lungs are called alveolar macrophages, whereas those in the capillaries of the lung are called pulmonary intravascular macrophages. Large numbers are found in the sinusoids of the spleen, bone marrow, and lymph nodes. Irrespective of their name or location, they are all macrophages and all are part of the mononuclear phagocyte system (Figure 2-5).

2.4.1.1 Structure

Macrophages change their shapes in response to their environment. In suspension, however, they are round cells about 15 μm in diameter. They possess abundant cytoplasm, at the center of which is a single nucleus that may be round, bean shaped, or indented (Figure 2-6). Their central cytoplasm contains mitochondria, large numbers of lysosomes, some rough endoplasmic reticulum, and a Golgi apparatus, indicating that they can synthesize and secrete proteins (Figures 2-7 and 2-8). In living cells, the peripheral cytoplasm is in continuous movement, forming and reforming veil-like ruffles. Some macrophages show variations from this basic structure. Peripheral blood monocytes have round nuclei, which elongate as the cells mature. Alveolar macrophages rarely possess rough endoplasmic reticulum, but their cytoplasm is full of granules. The

FIGURE 2-5 The location of the cells of the mononuclear phagocyte system.

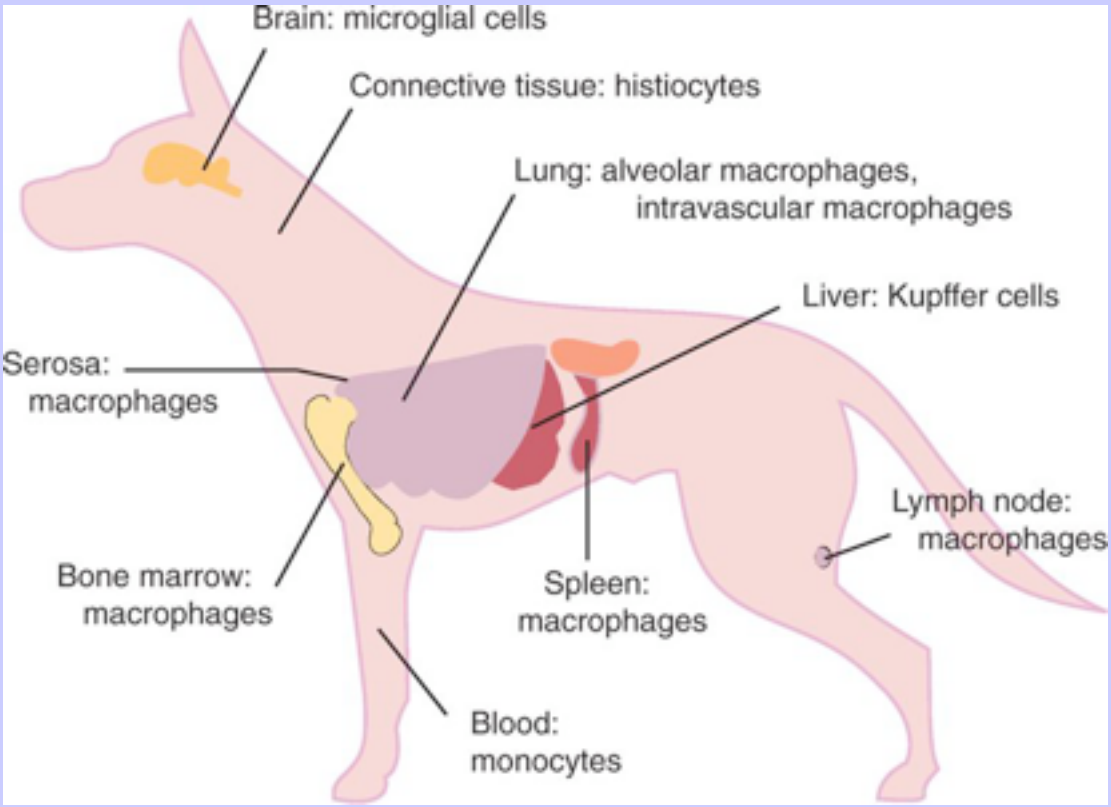


FIGURE 2-6 A typical macrophage, original magnification ×500.

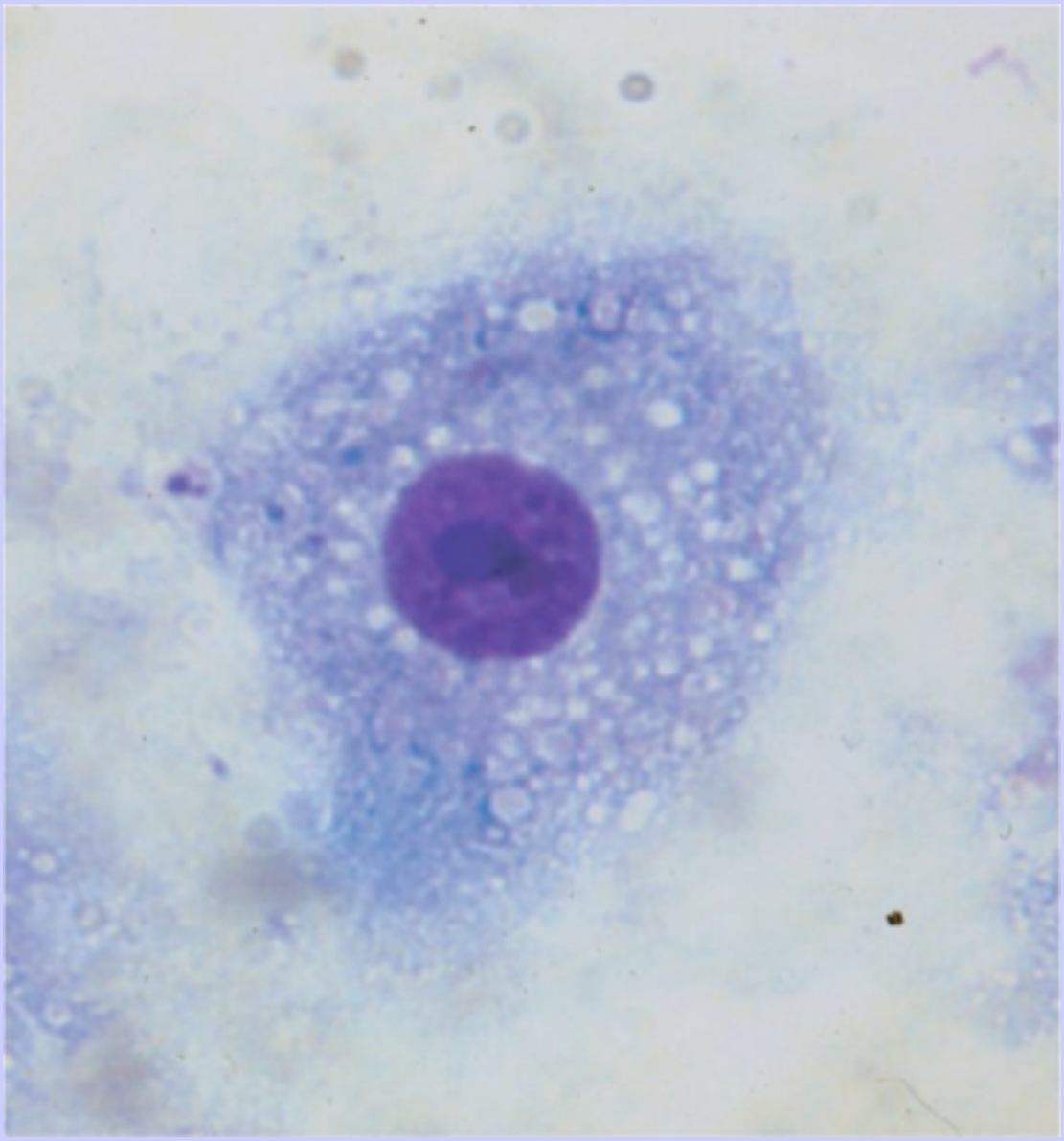


FIGURE 2-7 The major structural features of a macro-phage.

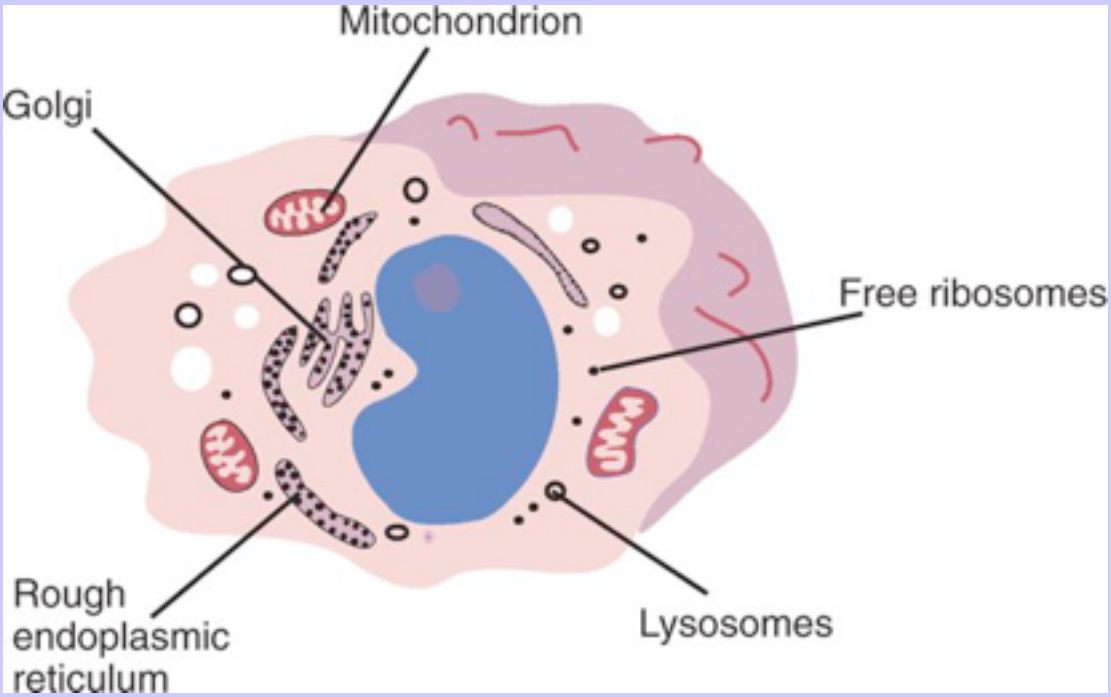


FIGURE 2-8 Transmission electron micrograph of a normal rabbit macrophage. The nature of the large inclusion is unknown. (Courtesy Dr. S. Linthicum.)

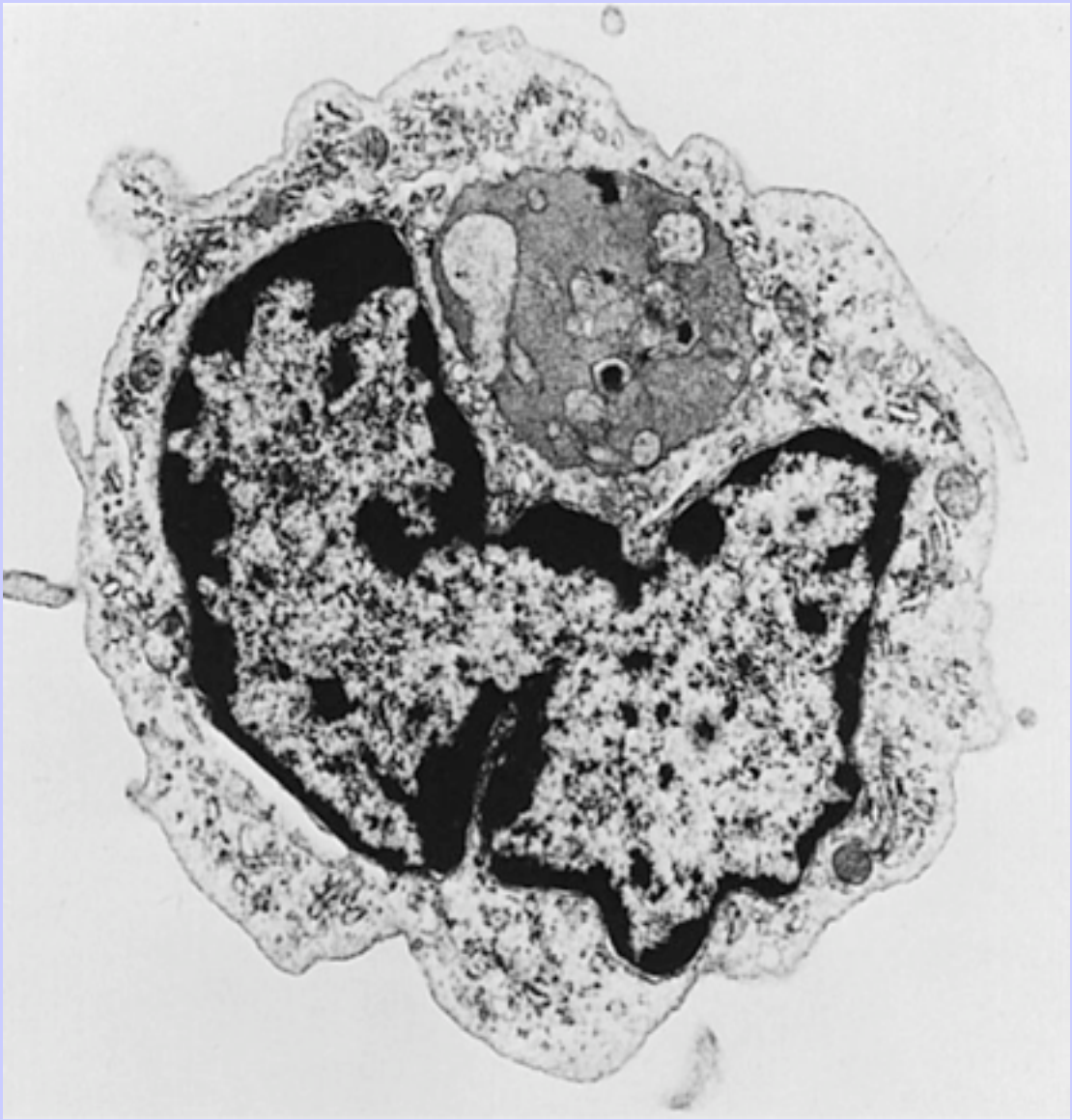
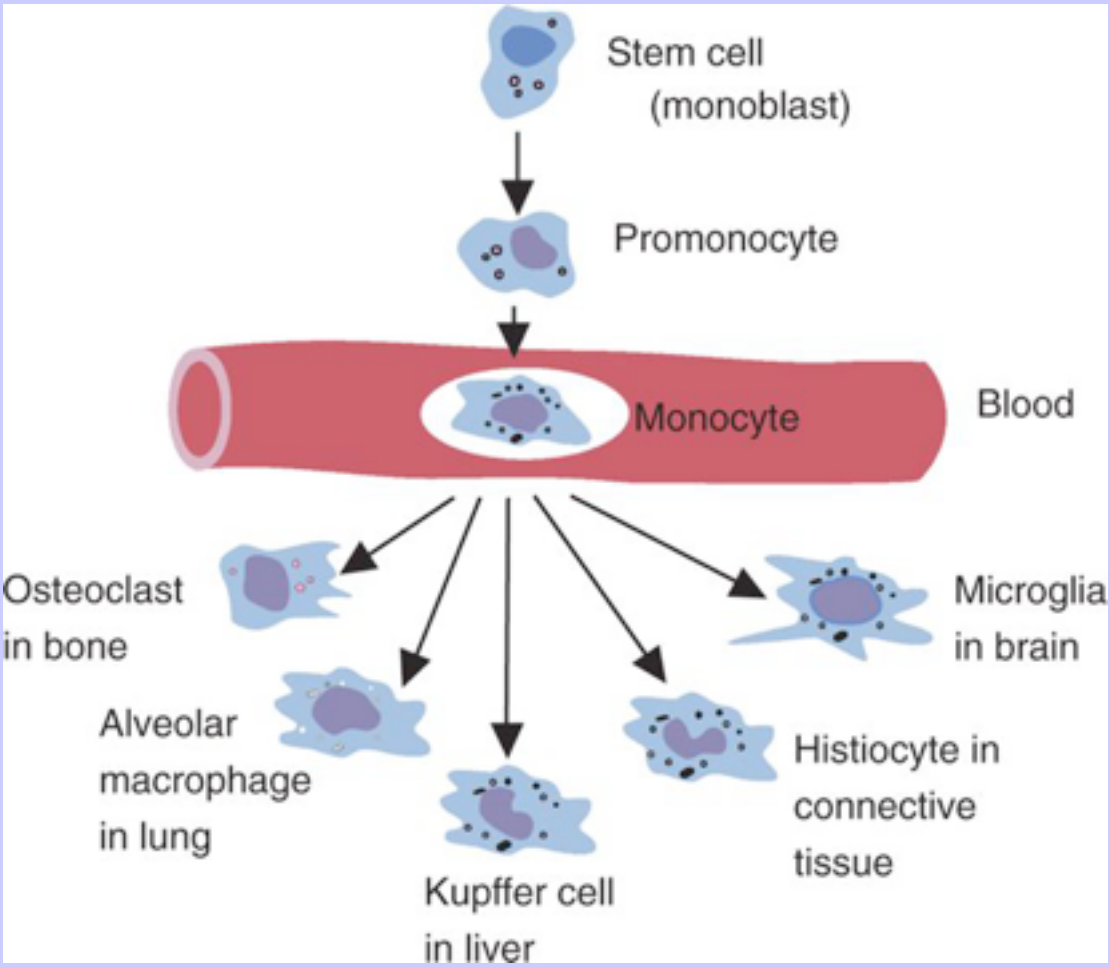


FIGURE 2-9 Origin and development of macrophages. Monocytes in blood can differentiate into many different types of macrophage.



microglia of the central nervous system have rod-shaped nuclei and very long cytoplasmic processes (dendrites) that are lost when the cell is stimulated by tissue damage.

2.4.1.2

Life History

All the cells of the mononuclear phagocyte system arise from stem cells in the bone marrow called monoblasts (Figure 2-9). Monoblasts develop into promonocytes, and promonocytes develop into monocytes, all under the influence of cytokines called colony-stimulating factors. Monocytes then enter the blood and circulate for about 3 days before entering tissues and developing into macrophages. They form about 5% of the total leukocyte population in blood. Tissue macrophages either originate from monocytes or divide within tissues. They are relatively long-lived cells, replacing themselves at a rate of about 1% per day unless activated by inflammation or tissue damage. Macrophages may live for a long time after ingesting chemically inert particles, such as the carbon injected in tattoo marks, although they may fuse together to form multinucleated giant cells in their attempts to eliminate the foreign material.

2.4.2 Dendritic Cells

The second population of sentinel cells consists of DCs—so called because many possess long, thin cytoplasmic processes called dendrites. DCs are a very heterogeneous population of cells but many are closely related to macrophages. They are discussed in detail in Chapter 8.

2.4.3 Mast Cells

2.4.3.1 Structure

Mast cells are large, round cells (15 to 20 μm in diameter) scattered throughout the body in connective tissue, under mucosal surfaces, in the skin, and around nerves (Figure 2-10). They are found in highest numbers at sites in the body exposed to potential invaders such as under the skin or in the intestine and airways. In these locations they are located close to

18

19

FIGURE 2-10 A section of canine skin stained to show mast cells. The mast cells stain intensely because of the heparin in their cytoplasmic granules.

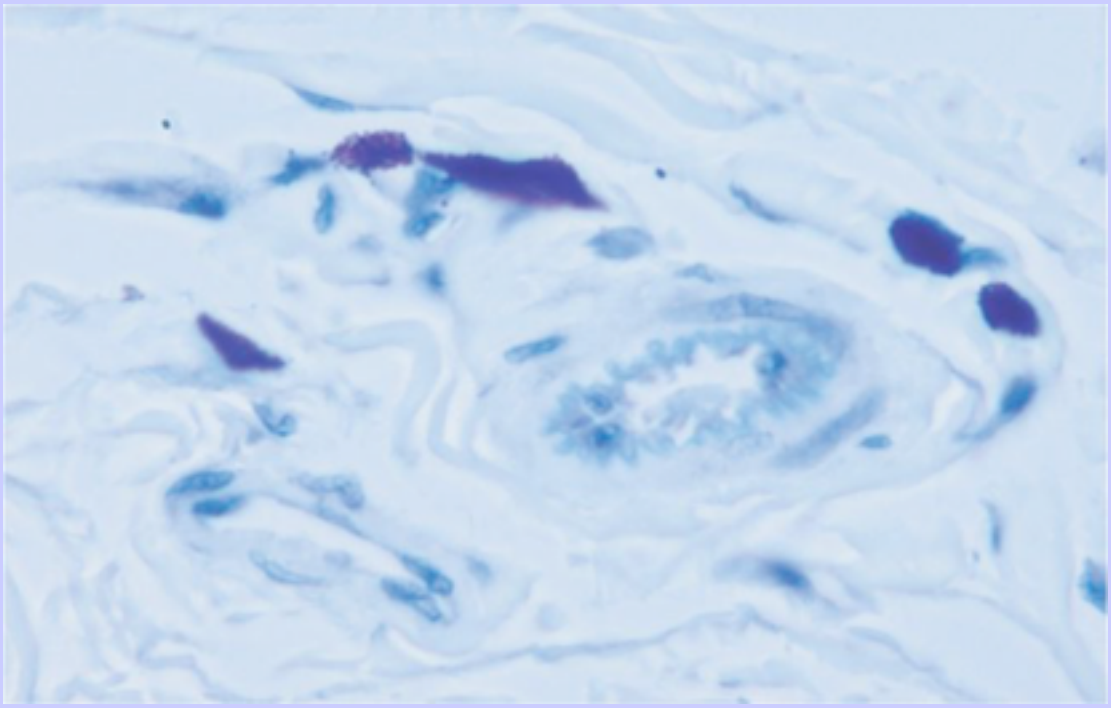
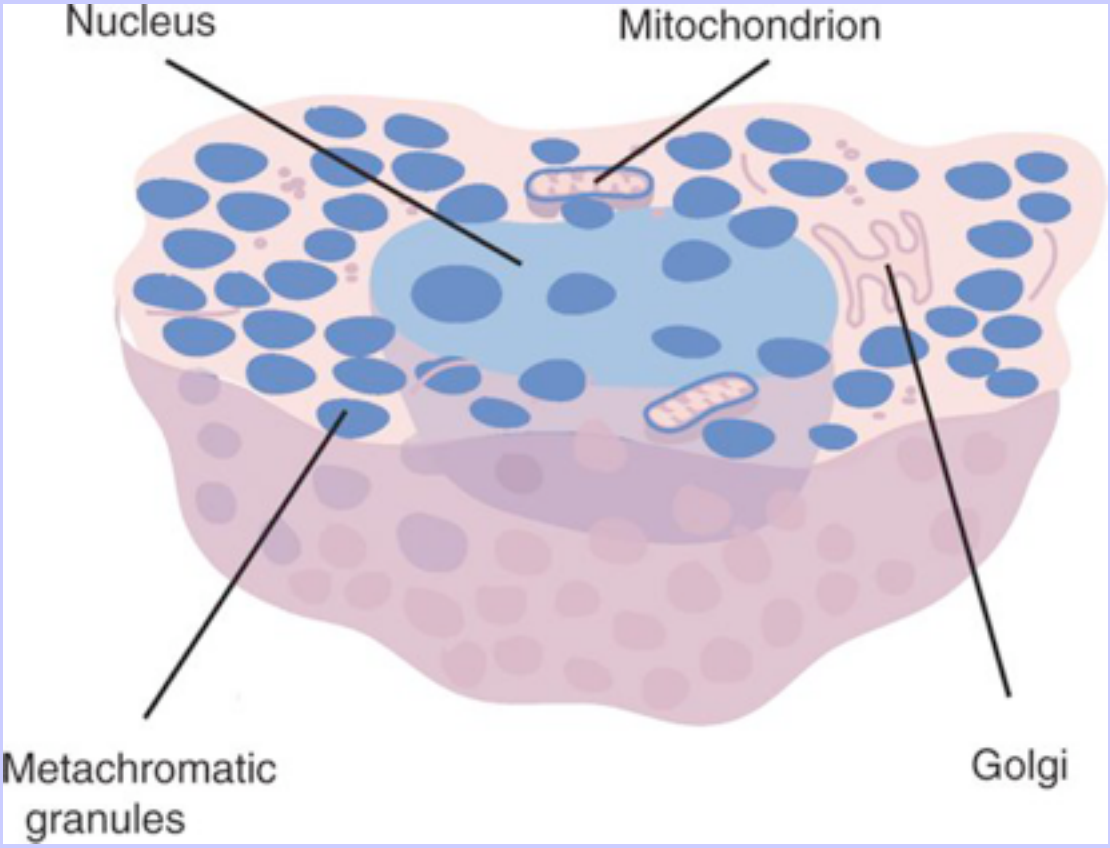


FIGURE 2-11 A diagram of the structural features of a connective tissue mast cell. The term metachromatic simply means that the granules stain intensely.



blood vessels, where they can regulate blood flow and influence cellular migration. They are easily recognizable because their cytoplasm is densely packed with large granules (secretory lysosomes) that stain very strongly with dyes such as toluidine blue. These granules often mask the large, bean-shaped nucleus (Figure 2-11). (Mast cells are so called because, being full of granules, they were considered to be "well-fed" cells (German *Mastzellen*). Mast cells from connective tissue and skin and from the intestinal walls differ both chemically and structurally (Table 2-3). For example, connective tissue and skin mast cells are rich in the molecules histamine and heparin, whereas mucosal mast cells contain chondroitin sulfate and have little histamine in their granules.

Table 2-3 Comparison of Two Major Types of Mast Cell

	Mucosal Mast Cells	Connective Tissue Mast Cells
Structure	Few, variable-sized granules	Many uniform granules
Size	9-10 μm diameter	19-20 m diameter
Proteoglycan	Chondroitin sulfate	Heparin
Histamine	1.3 pg/cell	15 pg/cell
Life span	<40 days	>6 mo
Location	Intestinal wall, lung	Peritoneal cavity, skin

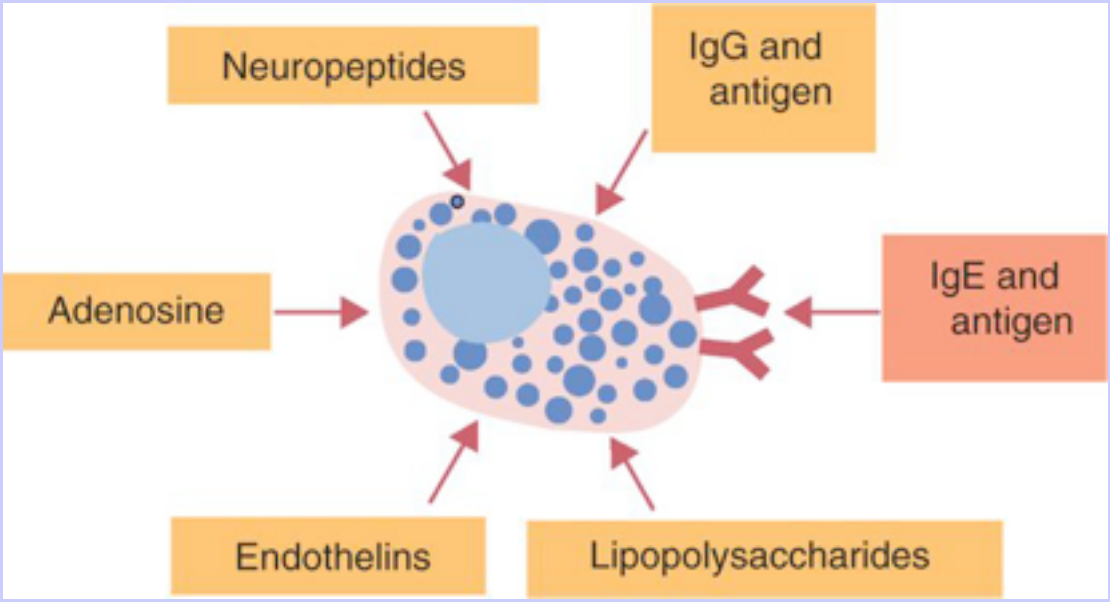
2.4.3.2

Life History

Mast cells originate from stem cells in the bone marrow. The mast cell precursors emigrate to tissues, where they mature and survive for several weeks or months. Although connective tissue mast cells remain at relatively constant levels, intestinal mast cells can proliferate. It has been suggested that the mucosal mast cells respond specifically to invasion by parasitic worms.

Mast cells have a key role in innate immunity because when they encounter invading microorganisms they release molecules that cause the changes in blood flow seen in acute inflammation. These inflammatory molecules are normally confined to the mast cell granules, but they are released when the cells degranulate. Many different mechanisms stimulate mast cell degranulation. The best recognized of these involves an antibody molecule called immunoglobulin E (IgE) (see Chapter 25). IgE and antigen together can trigger mast cell degranulation and so cause the severe inflammation that occurs in allergic diseases. However, allergies are a special case. Numerous other signals can activate mast cells including cytokines, chemokines, chemical agents, physical stimuli, various peptides, insect and animal venoms, bacteria and bacterial products, and viruses. In normal inflammation, mast cells release inflammatory mediators relatively slowly in a process called piecemeal degranulation. They may also secrete some vasoactive factors without degranulation (Figure 2-12). For example, bacteria and bacterial products can trigger mast cells to produce TNF-α, IL-1β, and IL-6 without degranulation. Many alarmins, including the defensins, neuropeptides, adenosine, and endothelins (small peptides from endothelial cells), also trigger mast cell degranulation.

FIGURE 2-12 Some of the stimuli that make mast cells degranulate. Antigen bound through immunoglobulin E (IgE) causes rapid, complete degranulation. The other stimuli shown cause a more gradual, piecemeal degranulation. Thus in normal inflammatory responses the degree of mast cell degranulation is tailored to local defensive needs.



Mast cells possess a wide array of pattern-recognition receptors that permit them to recognize the presence of pathogens. Thus they express TLRs 1, 2, 3, 4, 6, 7, and 9. They also possess a mannose receptor (CD48). As a result, mast cells can sense the presence of microbes and respond accordingly. Mast cells also possess receptors for molecules released by activation of the immune system such as some complement components.

Stimulation of their TLRs causes mast cells to release different mixtures of mediators. Thus bacterial peptidoglycans acting through TLR2 stimulate histamine release, whereas LPS acting through TLR4 do not. Mast cells can thus distinguish between different pathogens and generate highly selective combina-tions of cytokines, chemokines, and other inflammatory mediators, depending upon the stimulus they receive.

2.5 PRODUCTS OF SENTINEL CELLS

Macrophages, DCs, and mast cells are activated when PAMPs or alarmins bind to their receptors. As a result, they respond by synthesizing and secreting a mixture of cytokines and other molecules that trigger inflammation while starting to activate acquired immunity.

2.5.1 Cytokines

When exposed to infectious agents or their PAMPs, sentinel cells synthesize and secrete many different proteins including the major cytokines IL-1 and TNF-α, as well as IL-6, IL-12, and IL-18. They synthesize nitric oxide synthase 2 (NOS2), which generates oxidants such as nitric oxide. They synthesize the enzyme cyclooxygenase-2 (COX-2) that generates the inflammatory lipids prostaglandins and leukotrienes. When released in sufficient quantities, these molecules cause a fever and sickness behavior and promote an acute-phase response (see Chapter 4). If the sentinel cells detect the presence of damaged or foreign DNA, such as that from viruses, they trigger dendritic cells to secrete the antiviral cytokines known as interferons (see Chapter 23).

2.5.1.1 Tumor Necrosis Factor-a

TNF-α is a 25 kDa trimeric protein produced by macrophages, mast cells, T cells, endothelial cells, B cells, and fibroblasts. It can occur in soluble or membrane-bound forms. The membrane-bound form is cleaved from the cell surface by a protease called TNF-α convertase. TNF-α plays a key role in triggering inflammation. Upon detecting invading pathogens, macrophages and mast cells secrete either membrane-associated TNF-α or soluble TNF-α. The TNF-α triggers local release of chemokines and cytokines and promotes the adherence, migration, attraction, and activation of leukocytes at the site of invasion. Later, TNF-α facilitates the transition from innate to acquired immunity by enhancing antigen presentation and T cell co-stimulation. Its production is stimulated not only through TLRs but also by molecules secreted by nerves such as the neurotransmitter substance P. TNF-α is produced very early in inflammation, and this is followed by waves of IL-1 and then by IL-6.

TNF-α is an essential mediator of inflammation because in combination with IL-1 it triggers changes in the cells that line small blood vessels (vascular endothelial cells). A local increase in TNF-α causes the “cardinal signs” of inflammation, including heat, swelling, pain, and redness. Systemic increases in TNF-α depress cardiac output, induce microvascular thrombosis, and cause capillary leakage. TNF-α acts on neutrophils (key defensive cells in inflammation; see Chapter 3) to enhance their ability to kill microbes. It attracts neutrophils to sites of tissue damage and increases their adherence to vascular endothelium (Figure 2-13). It stimulates macrophage phagocytosis and oxidant production. It amplifies and prolongs inflammation by promoting macrophage synthesis of IL-1, NOS2, and COX-2. TNF-α also activates mast cells.

TNF-α activates macrophages to increase its own synthesis together with that of IL-1. As its name implies, TNF-α can kill some tumor cells and virus-infected cells by activating caspases and inducing apoptosis. In high doses, TNF-α can cause septic shock.

2.5.1.2 Interleukin-1

When stimulated by CD14 and TLR4, macrophages synthesize two glycoproteins called IL-1α and IL-1β. IL-1β is produced as a large pro-protein that is cleaved

FIGURE 2-13 Some of the properties of tumor necrosis factor-α.

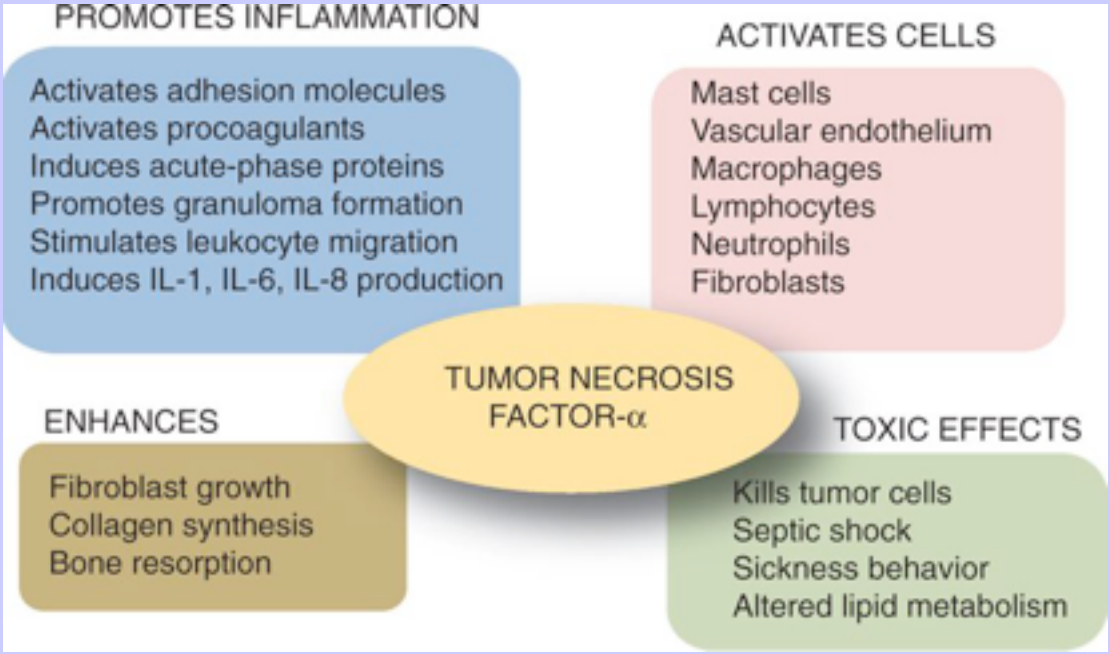
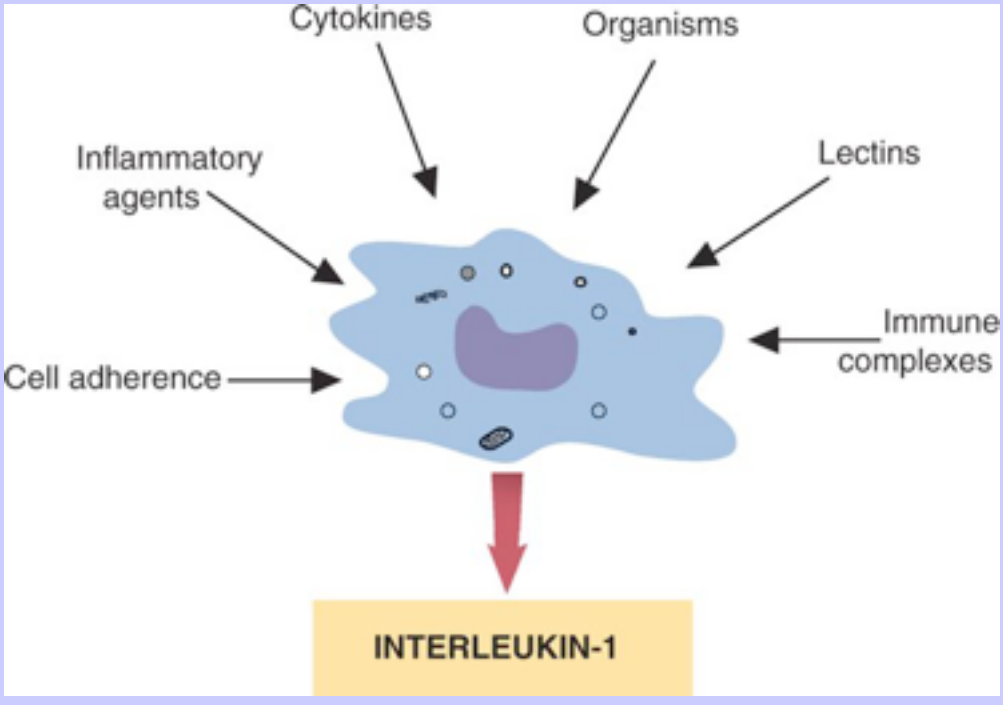


FIGURE 2-14 The wide variety of stimuli that promote the release of interleukin-1 from macrophages.



by caspase-1 to form the active molecule. Tenfold to fiftyfold more IL-1β is produced than IL-1α, and while IL-1β is secreted, IL-1α remains attached to the cell. Therefore IL-1α acts only on target cells that come into direct contact with the macrophage (Figure 2-14). Transcription of IL-1β mRNA occurs within 15 minutes of ligand binding. It reaches a peak 3 to 4 hours later and levels off for several hours before declining. Like TNF-α, IL-1β acts on vascular endothelial cells to make them adhesive for neutrophils. IL-1 acts on other macrophages to stimulate their synthesis of NOS2 and COX-2 and so promote more inflammation.

During severe infections, some IL-1β circulates in the bloodstream, where (in association with TNF-α) it is responsible for sickness behavior. Thus it acts on the brain to cause fever, lethargy, malaise, and lack of appetite (Figure 2-15). It acts on muscle cells to mobilize amino acids causing pain and fatigue. It acts on liver cells to induce the production of new proteins, called acute-phase proteins, that assist in the defense of the body (see Chapter 4).

The most important IL-1 receptors are CD121a and CD121b. CD121a is a signaling receptor, whereas CD121b is not. CD121b thus inhibits IL-1 functions. Soluble CD121b can bind IL-1 and so acts as an IL-1 antagonist. IL-1 receptor antagonist (IL-1RA) is an inactive molecule that binds and blocks CD121a. IL-1RA is therefore an important regulator of IL-1 activity and inflammation. It reduces mortality in septic shock and graft-versus-host disease (see Chapter 4) and has antiinflammatory effects.

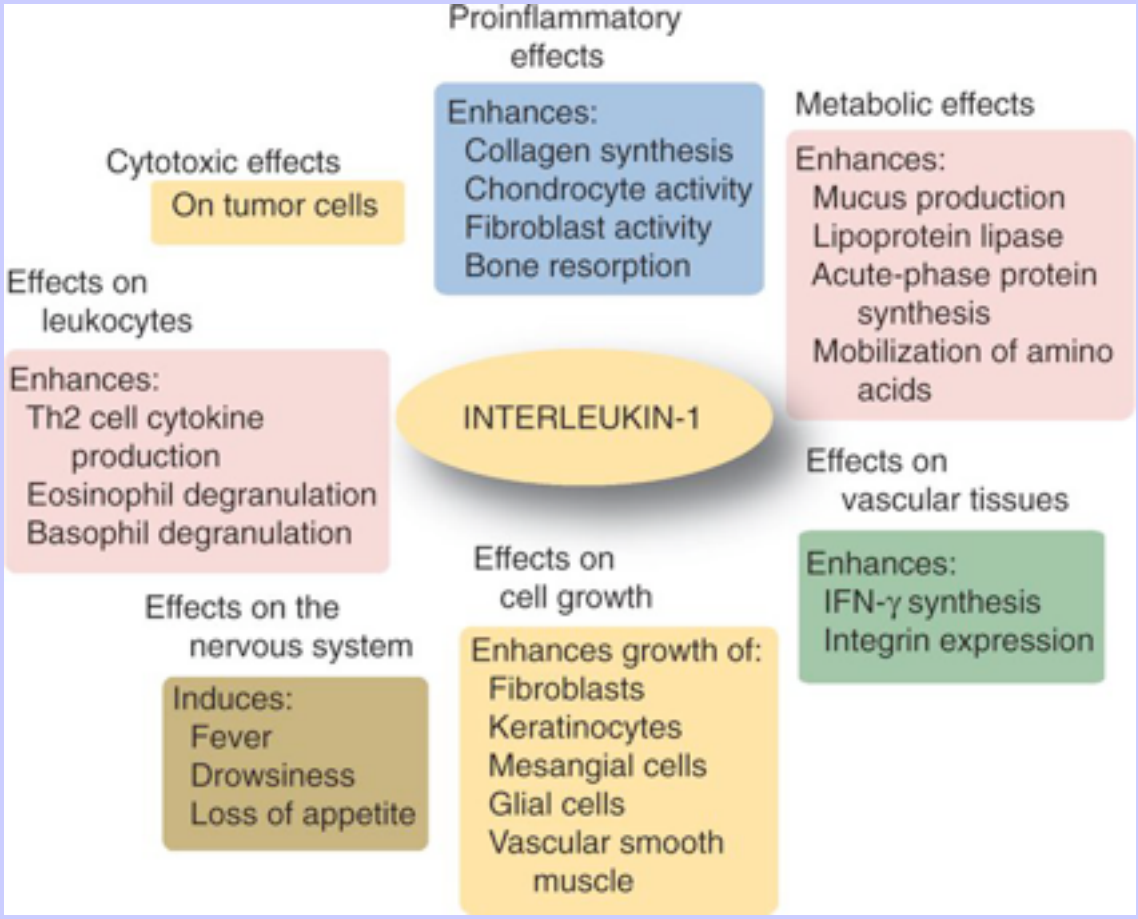
2.5.1.3 Interleukin-6

IL-6 is also produced by macrophages and mast cells. Its production is stimulated by the bacterial endotoxins, IL-1 and TNF-α. IL-6 affects both inflammation and acquired immunity. It is a major mediator of the acute-phase reaction and of septic shock (see Chapter 4). It has been suggested that IL-6 regulates the transition from a neutrophil-dominated process early in inflammation to a macrophage-dominated process later on.

2.5.2 Chemokines

Chemokines are a family of small (8 to10 kDa) proteins that control cellular migration. Because they regulate the movement of specific cell populations, they can dictate the course of many inflammatory and immune responses (Table 2-4). Chemokines are produced by diverse cell types including macrophages and mast cells. At least 50 different chemokines have been identified. They are classified into four families according to the spacing of their cysteine residues (Figure 2-16). For example, the CC, or a, chemokines have two contiguous cysteine residues, whereas the CXC, or b, chemokines have two cysteine residues separated by another amino acid. (Chemokine nomenclature is based on this classification, each molecule or receptor receiving a numerical designation. Ligands have the

FIGURE 2-15 Some of the effects of interleukin-1 on the cells of the body.



suffix “L” [e.g., CXCL8], whereas receptors have the suffix “R” [e.g., CXCR1].)

Table 2-4 The Nomenclature of Some Selected Chemokines and Their Receptors

New Name	Old Name	Receptor
α Family		
CCL2	MCP-1	CCR2
CCL3	MIP-1α	CCR1, CCR5
CCL4	MIP-1β	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
CCL7	MCP-3	CCR3
CCL8	MCP-2	CCR3
CCL11	Eotaxin	CCR3
CCL13	MCP-4	CCR3
CCL20	MIP-3α	CCR6
CCL22	MDC	CCR4
CCL26	Eotaxin 3	CCR3
CCL28	MEC	CCR3
β Family		
CXCL1	GRO1	CXCR2
CXCL7	MDGF	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL12	SDF	CXCR4
CXCL13	BCA-1	CXCR5
γ Family		
XCL1	Lymphotactin	XCR1
δ Family		
CX3CL1	Fractalkine	CX3CR1

CXCL8 (or IL-8) is a typical example of a CXC chemokine produced by stimulation of macrophages or mast cells. CXCL8 will attract and activate neutrophils, releasing their granule contents and stimulating the respiratory burst and leukotriene release (see Chapter 3). Another important CXC chemokine is CXCL2 (macrophage inflammatory protein-2, MIP-2), which is sec-reted by macrophages and also attracts neutrophils.

CC chemokines act predominantly on macrophages and DCs. Thus CCL3 and CCL4 (MIP-1a and MIP-1b) are produced by macrophages and mast cells. CCL4 attracts CD4+ T cells, whereas CCL3 attracts B cells, eosinophils, and cytotoxic T cells. CCL2 (monocyte chemotactic protein-1, MCP-1) is produced by macrophages, T cells, fibroblasts, keratinocytes, and endothelial cells. It attracts and activates monocytes, stimulating their

respiratory burst and lysosomal enzyme release. CCL5 (RANTES [regulated on activation normal T cell expressed and secreted]) is produced by T cells and macrophages. It is chemotactic for monocytes, eosinophils, and some T cells. It activates eosinophils and stimulates histamine release from basophils. Regakine-1 is a CC chemokine found in bovine serum that acts together with CXCL8 and C5a to attract neutrophils and enhance inflammation.

Two chemokines fall outside the CC and CXC families. A C (only one cysteine residue) or γ chemokine, called XCL1 (or lymphotactin), is chemotactic for lymphocytes. Its receptor is XCR1. The CXXXC (two cysteines separated by three amino acids) or δ chemokine called CX3CL1 (or fractalkine) triggers adhesion by T cells and monocytes. Its receptor is CX3CR1.

22

23

Most chemokines are produced in inflamed or damaged tissues and attract other cells to sites of inflammation or microbial invasion. It is probable that several different chemokines serve to attract different cell types to inflammatory sites. Indeed it is likely that the chemokine mixture produced in damaged tissues regulates the precise composition of the inflammatory cell populations. In this way the body can adjust the inflammatory response to provide the most effective way of destroying different microbial invaders. Many chemokines, such as CXCL4, CCL20, and CCL5, are structurally similar to the antimicrobial proteins called defensins and, like them, have significant antibacterial activity. Chemokines have a major role in infections and inflammation in domestic animal species. They regulate immune cell trafficking. They have been detected in many inflammatory diseases, including pneumonia (bovine pasteurellosis), bacterial mastitis, arthritis, and endotoxemia. Impaired neutrophil migration is associated with specific CXCR2 genotypes and may lead to increased susceptibility to bovine mastitis.

2.6 INCREASED VASCULAR PERMEABILITY

Acute inflammation can develop within minutes after a tissue is damaged. The damaged tissue triggers three types of signal. First, broken cells release alarmins that trigger the release of cytokines from sentinel cells. Second, microbes provide PAMPs that trigger sentinel cell responses, including the production of cytokines and other inflammatory mediators. Third, pain causes nerves to release bioactive peptides.

In its classical form, acute inflammation has five cardinal signs: heat, redness, swelling, pain, and loss of function. All these signs result from changes in small blood vessels (Figure 2-17). Immediately after injury the blood flow through small capillaries at the injection site is decreased to give leukocytes an opportunity to bind to the blood vessel walls. Shortly thereafter, the small blood vessels in the damaged area dilate and blood flow to the injured tissue increases. While the blood vessels are dilated, they also leak so that fluid moves from the blood into the tissues where it causes edema and swelling.

At the same time as these changes in blood flow are occurring, cellular responses are taking place. The changes in cells lining blood vessel walls permit neutrophils and monocytes to adhere to the vascular endothelial cells. If the blood vessels are damaged, blood platelets may also bind to the injured sites and release vasoactive and clotting molecules.

Inflamed tissues swell as a result of leakage of fluid from blood vessels. This leakage occurs in two stages. First there is an immediate increase in leakage mediated by vasoactive molecules released by mast cells, by damaged tissues, and by nerves (Table 2-5). The second phase of increased leakage occurs several hours after the onset of inflammation, at a time when the leukocytes are beginning to emigrate. Endothelial and perivascular cells contract so that they pull apart and allow fluid to escape through the intercellular spaces.

2.7 VASOACTIVE MOLECULES

Mast cells respond to signals from damaged tissues by releasing a mixture of molecules that affect blood vessel walls (vasoactive molecules). These include histamine, vasoactive lipids, enzymes (tryptase and

FIGURE 2-16 The classification of chemokines is based on the location and spacing of their cysteine residues.

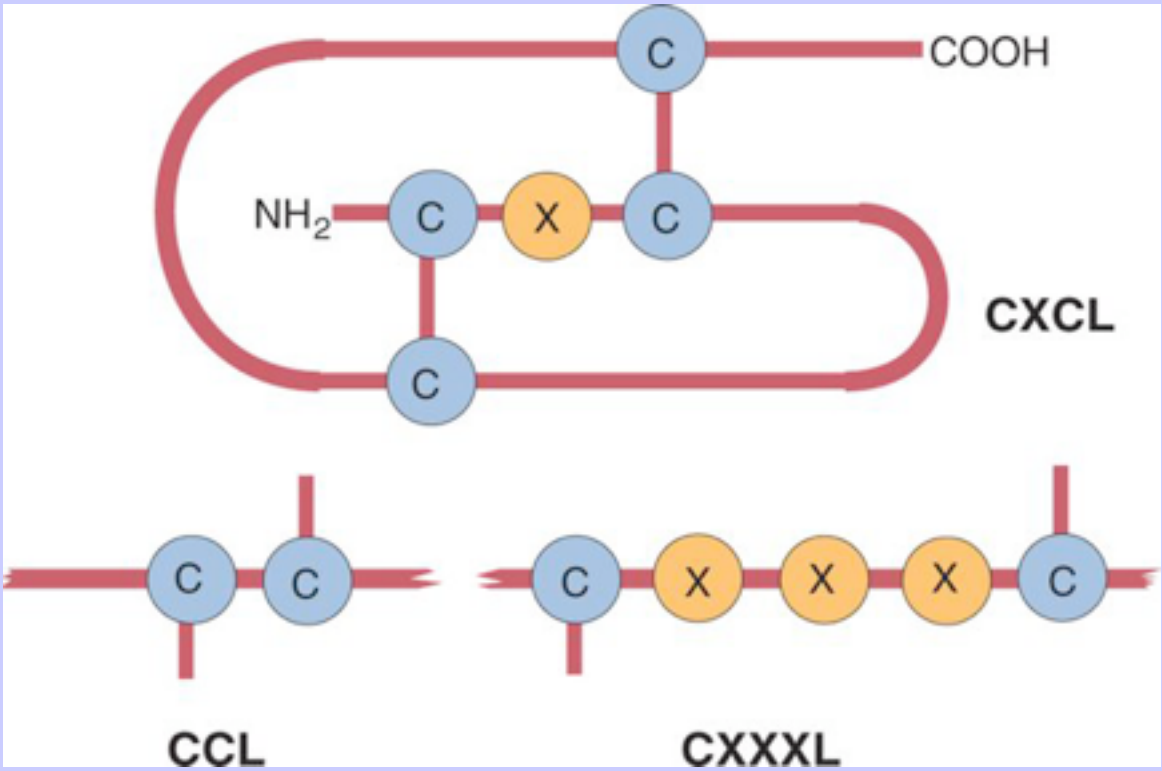
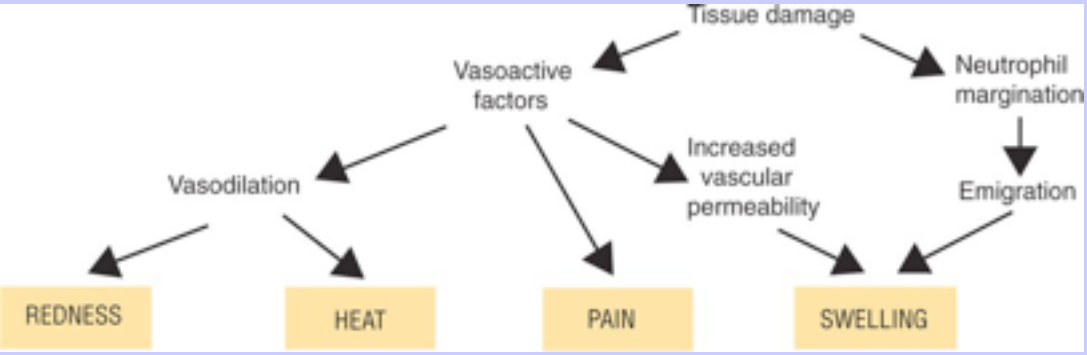


FIGURE 2-17 The cardinal signs of acute inflammation and how they are generated.



chymase), cytokines, and chemokines. Histamine, the lipids, and tryptases cause vasodilation and blood vessel leakage of fluid. The tryptases activate receptors on mast cells, sensory nerve endings, vascular endothelial cells, and neutrophils. As a result, the blood vessel walls become sticky for neutrophils. Activated neutrophils release a lipid called platelet-activating factor (PAF). The PAF makes endothelial cells even stickier and so enhances neutrophil adhesion and emigration. PAF is a phospholipid closely related to lecithin. It is synthesized by mast cells, platelets, neutrophils, and eosinophils. PAF aggregates platelets and makes them release their vasoactive molecules and synthesize thromboxanes. It acts on neutrophils in a similar fashion. Thus it promotes neutrophil aggregation, degranulation, chemotaxis, and release of oxidants.

Table 2-5 Some Vasoactive Molecules Produced during Acute Inflammation

Mediator	Major Source	Function
Histamine	Mast cells and basophils, platelets	Increased vascular permeability, pain
Serotonin	Platelets, mast cells, basophils	Increased vascular permeability
Kinins	Plasma kininogens and tissues	Vasodilation
		Increased vascular permeability, pain
Prostaglandins	Arachidonic acid	Vasodilation, increased vascular permeability
Thromboxanes	Arachidonic acid	Increased platelet aggregation
Leukotriene B_4	Arachidonic acid	Neutrophil chemotaxis
		Increased vascular permeability
Leukotrienes C, D, E	Arachidonic acid	Smooth muscle contraction
		Increased vascular permeability
Platelet-activating factor	Phagocytic cells	Platelet secretion
		Neutrophil secretion
		Increased vascular permeability
Fibrinogen breakdown products	Clotted blood	Smooth muscle
		Neutrophil chemotaxis
		Increased vascular permeability
C3a and C5a	Serum complement	Mast cell degranulation
		Smooth muscle contraction
		Neutrophil chemotaxis (C5a)

The most important of the vasoactive molecules released by mast cells is histamine (Figure 2-18). The effects of histamine are mediated through multiple receptors. H1 and H2 receptors are expressed on nerve cells, smooth muscle cells, endothelial cells, neutrophils, eosinophils, monocytes, DCs, and T and B cells. Histamine binding to H1 receptors stimulates endothelial cells to produce nitric oxide, a very potent vasodilator. At the same time, histamine causes vascular leakage, leading to fluid accumulation and local edema. Histamine upregulates TLR expression on sentinel cells.

Serotonin (5-hydroxytryptamine [5-HT]), a derivative of the amino acid tryptophan, is released from the mast cells of some rodents and the large domestic herbivores. Serotonin normally causes a vasoconstriction that results in a rise in blood pressure (except in cattle, in which it is a vasodilator). It has little effect on vascular permeability, except in rodents, where it induces acute inflammation.

Although histamine and serotonin are very important mediators of inflammation, they are only a fraction of the complex mixture of molecules released when mast cells degranulate. More than half the protein in mast cell granules consists of proteases called tryptases and chymases. These enzymes sensitize smooth muscle to histamine; they stimulate proliferation of fibroblasts, smooth muscle, and epithelial cells; generate kinins; increase expression of adherence proteins; and stimulate the release of the chemokine CXCL8. Mast cell tryptases can also activate some receptors on sensory nerves, neutrophils, mast cells, and endothelial cells.

2.7.1 Vasoactive Lipids

When tissues are damaged or stimulated, phospholipases act on cell wall phospholipids to release arachidonic acid. Under the influence of the enzyme 5-lipoxygenase, the arachidonic acid is converted to biologically active lipids called leukotrienes (Figure 2-19). 24 25

FIGURE 2-18 Structure of some major vasoactive molecules active during acute inflammation.

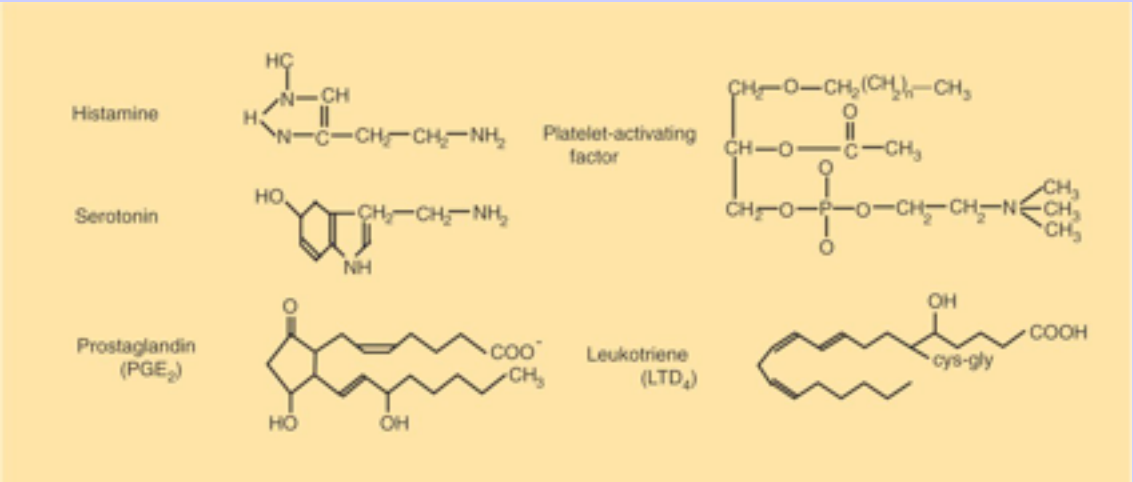
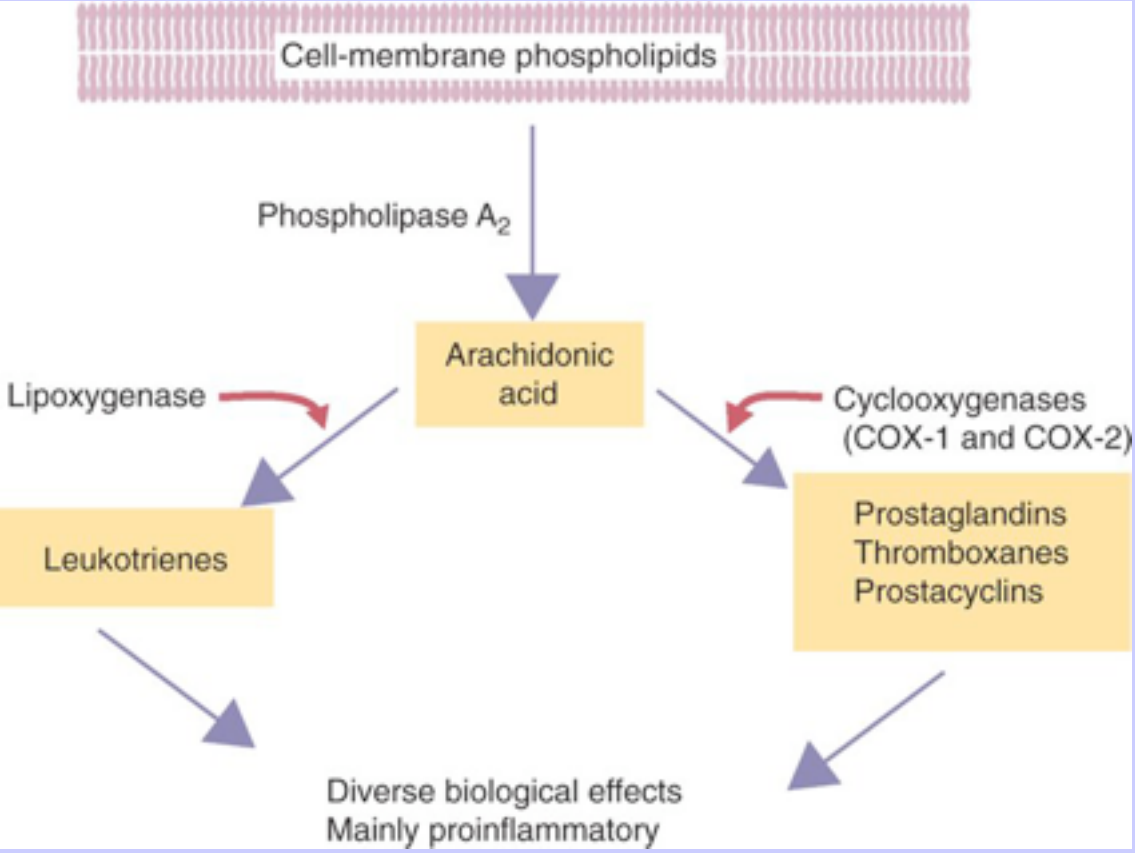


FIGURE 2-19 Production of leukotrienes and prostaglandins by the actions of lipoxygenase and cyclooxygenase (COX) on arachidonic acid. Both prostaglandins and leukotrienes may have proinflammatory or antiinflammatory activity depending on their chemical structure.



Under the influence of cyclooxygenases, arachidonic acid is converted to a second group of active lipids called prostaglandins. The collective term for all these complex lipids is eicosanoids.

Four leukotrienes play a central role in inflam-mation. Leukotriene B_4 (LTB_4) is an especially potent neutrophil attractant and is probably one of the most important mediators released by mast cells during bacterial infections. LTB_4 also stimulates eosinophil chemotaxis and random motility. Leukotrienes C_4, D_4, and E_4, in contrast, increase vascular permeability.

There are four groups of proinflammatory prostaglandins: PGE_2, PGF_2, the thromboxanes (TxA_2, PGA_2), and the prostacyclins (PGI_2). The enzymes that generate the prostacyclins are found in vascular endothelial cells, the thromboxanes are found in platelets, and the other prostaglandins can be generated by most nucleated cells. The biological activities of the prostaglandins vary widely, and since many different prostaglandins are released in inflamed tissues, their net effect may be very complex.

As neutrophils enter inflammatory sites, they use the enzyme 15-lipoxygenase to produce lipoxins from arachidonic acid. These oxidized eicosanoids bind cellular receptors and inhibit neutrophil migration. Thus there is a gradual switch in production from proinflammatory leukotrienes to antiinflammatory lipoxins. The rise in PGE_2 in tissues also gradually inhibits 5-lipoxygenase activity and so eventually suppresses inflammation.

2.7.2 Vasoactive Peptides

Mast cell proteases act on the complement components C3 and C5 to generate two small, biologically active peptides—C3a and C5a (see Chapter 5). Both promote histamine release from mast cells. In addition, C5a is a very potent attractant for neutrophils and monocytes. Mast cell granules also contain proteases called kallikreins. These act on proteins called kininogens to generate small peptides called kinins. Both the kinins and the anaphylatoxins cause blood vessel dilation and leakage. The most important of the kinins is bradykinin. Kinins not only increase vascular permeability, they also stimulate neutrophils and trigger pain receptors and they may have defensin-like antimicrobial activity.

2.8 THE COAGULATION SYSTEM

When fluid leaks from the bloodstream into the tissues, blood coagulation is activated. Platelet aggregation accelerates this process. Activation of the coagulation system generates large quantities of thrombin, the main clotting enzyme. Thrombin acts on fibrinogen in tissue fluid and plasma to produce insoluble fibrin. Fibrin is therefore deposited in inflamed tissues, where it forms an effective barrier to the spread of infection. Activation of the coagulation cascade also initiates the fibrinolytic system. This leads to activation of plasminogen activator, which in turn generates plasmin, a potent fibrinolytic enzyme. In destroying fibrin, plasmin releases peptide fragments that are chemotactic for neutrophils.

2.9 SOURCES OF ADDITIONAL INFORMATION

P Ahmad-Nejad, H Häcker, M Rutz, et al.: Bacterial CpG-DNA and lipopolysaccharides activate toll-like receptors at distinct cellular compartments. *Eur J Immunol*. **32**, 2002, 1958–1968.

S Akira: Mammalian toll-like receptors. *Curr Opin Immunol*. **15**, 2003, 5–11.

U Andersson, H Erlandsson-Harris, H Yang, KJ Tracey: HMGB1 as a DNA-binding cytokine. *J Leukoc Biol*. **72**, 2002, 1084–1091.

M Baggiolini: Chemokines and leukocyte traffic. *Nature*. **392**, 1998, 565–568.

M Baggiolini: Chemokines in pathology and medicine. *J Intern Med*. **250**, 2001, 91–104.

AA Beg: Endogenous ligands of toll-like receptors: implications for regulating inflammatory and immune responses. *Trends Immunol*. **23**, 2002, 509–511.

K Dabbagh, ME Dahl, P Stepick-Biek, DB Lewis: Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol*. **168**, 2002, 4524–4530.

V Gangur, NP Birmingham, S Thanesvorakul: Chemokines in health and disease. *Vet Immunol Immunopathol*. **86**, 2002, 127–136.

S Gordon: Pattern recognition receptors: doubling up on the innate immune response. *Cell*. **111**, 2002, 927–930.

K Heeg, S Zimmerman: CpG DNA as a Th1 trigger. *Int Arch Allergy Immunol*. **121**, 2000, 87–97.

F Heil, H Hochrein, F Ampenberger, et al.: Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*. **303**, 2004, 1526–1529.

R Higgs, P Cormican, S Cahalane, et al.: Induction of a novel chicken toll-like receptor following Salmonella enterica Serovar Typhimurium infection. *Infect Immun*. **74**, 2006, 1692–1698.

GB Johnson, GJ Brunn, Y Kodaira, JL Platt: Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulfate by toll-like receptor 4. *J Immunol*. **168**, 2002, 5233–5239.

G Kaplanski, V Marin, F Montero-Julian, et al.: IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends Immunol*. **24**, 2003, 25–30.

AM Krieg: Now I know my CpGs. *Trends Microbiol*. **9**, 2001, 249–251.

R Malaviya, SN Abraham: Mast cell modulation of immune responses to bacteria. *Immunol Rev*. **179**, 2001, 16–24.

JS Marshall: Mast cell responses to pathogens. *Nat Rev Immunol*. **4**, 2004, 787–799.

R Medzhitov, C Janeway, Jr.: Innate immunity. *N Engl J Med*. **343**, 2000, 338–343.

M Menzies, A Ingham: Identification and expression of Toll-like receptors 1-10 in selected bovine and ovine tissues. *Vet Immunol Immunopathol*. **109**, 2006, 23–30.

LA O'Neill: Toll-like receptor signal transduction and the tailoring of innate immunity: a role for Mal?. *Trends Immunol*. **23**, 2002, 296–300.

M Rehli: Of mice and men: species variations of toll-like receptor expression. *Trends Immunol*. **23**, 2002, 375–378.

Y Sang, B Ramanathan, CR Ross, F Blecha: Gene silencing and overexpression of porcine peptidoglycan recognition protein long isoforms: involvement in b-defensin-1 expression. *Infect Immun*. **73**, 2005, 7133–7141.

M Stassen, L Hultner, E Schmitt: Classical and alternative pathways of mast cell activation. *Crit Rev Immunol*. **22**, 2002, 115–140.

H Strom, HK Thomsen: Effects of proinflammatory mediators on canine neutrophil chemotaxis and aggregation. *Vet Immunol Immunopathol*. **25**, 1990, 209–218.

O Takeuchi, S Sato, T Horiuchi, et al.: Role of toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol*. **169**, 2002, 10–14.

J Talreja, MK Kabir, MB Filla, et al.: Histamine induces toll-like receptor 2 and 4 expression in endothelial cells and enhances sensitivity to Gram-positive and Gram-negative bacterial cell wall components. *Immunology*. **113**, 2004, 224–233.

CC Tydell, J Yuan, P Tran, ME Selsted: Bovine peptidoglycan recognition protein-S: Antimicrobial activity, localization, secretion, and binding properties. *J Immunol*. **176**, 2006, 1154–1162.

H Wagner: The immunobiology of the TLR9 subfamily. *Trends Immunol*. **25**, 2004, 381–386.

F Yarovinsky, D Zhang, JF Anderson, et al.: TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science*. **308**, 2005, 1626–1629.

D Zhang, G Zhang, MS Hayden, et al.: A toll-like receptor that prevents infection by uropathogenic bacteria. *Science*. **303**, 2004, 1522–1526.

FG Zhu, JS Marshall: CpG-containing oligodeoxynucleotides induce TNF-alpha and IL-6 production but not degranulation from murine, bone-marrow derived mast cells. *J Leukoc Biol*. **69**, 2001, 253–262.