Veterinary Immunology: An Introduction, 8th Edition

4 CHAPTER 4 Macrophages and the Later Stages of Inflammation

4.1 KEY POINTS

- Macrophages move into sites of inflammation after neutrophils. They ingest and kill surviving microbial invaders.

- Macrophages ingest dead and dying neutrophils and so limit the damage caused by escaping neutrophil enzymes.

- Macrophages generate the powerful oxidizing agent nitric oxide.

- Macrophages effectively remove foreign particles from the bloodstream and the respiratory tract.

- Macrophages are responsible for beginning the healing process in damaged tissues.

- Cytokines secreted by sentinel cells cause a fever and are responsible for the behavioral changes that we call sickness.

- Excessive production of these cytokines (a cytokine storm) can lead to lethal shock syndromes.

- Excessive, chronic release of inflammatory cytokines can cause deposition of insoluble proteins called amyloid in tissues.
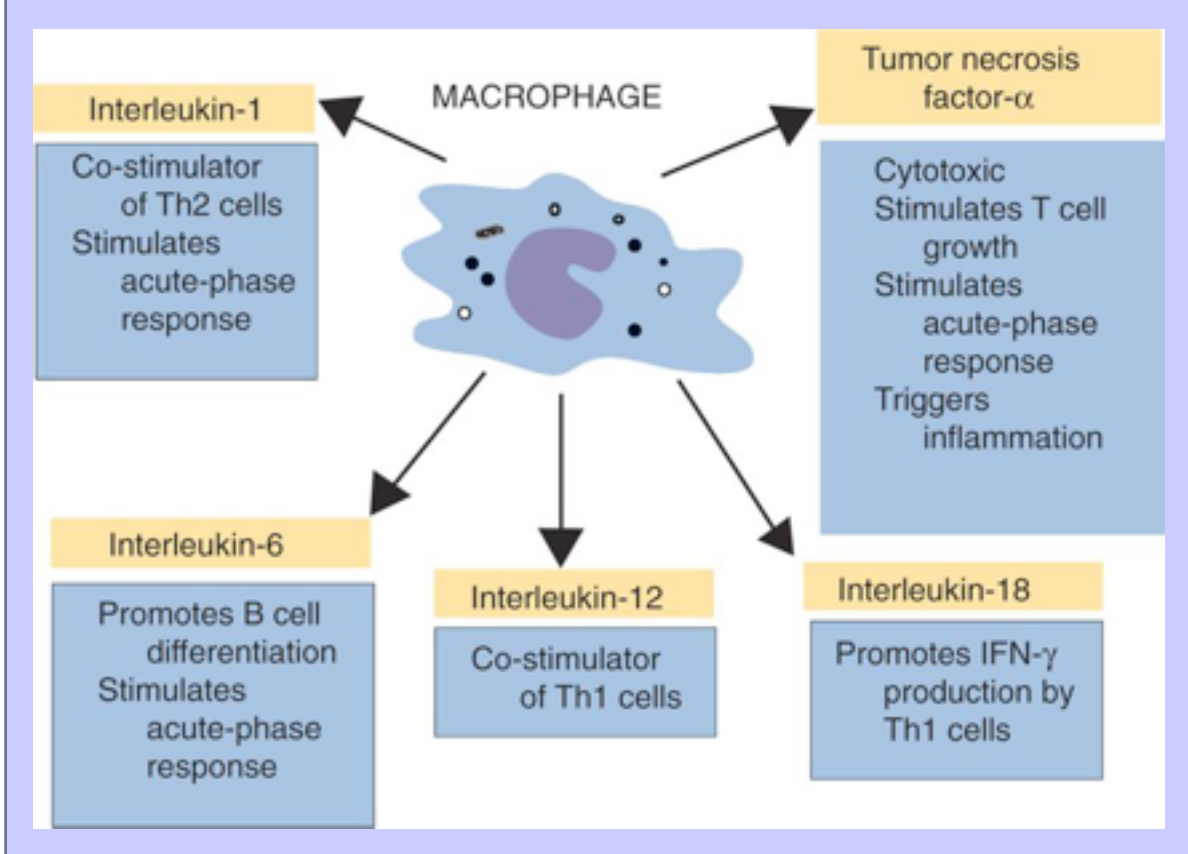
Although neutrophils act as a first line of defense, mobilizing rapidly and eating and killing invading microorganisms with enthusiasm, they cannot by themselves ensure that all invaders are killed. The body therefore employs a "backup" system employing phagocytic cells called monocytes (when in the bloodstream) and macrophages (when in tissues). Macrophages differ from neutrophils in their speed of response, which is slower; in their antimicrobial abilities, which are greater; and in their ability to stimulate acquired immune responses. Unlike neutrophils, which are specialized for the single task of killing invading organisms, macrophages have diverse functions. These include not only triggering inflammation by acting as sentinel cells, but also cleaning up the mess afterward.

4.2 MACROPHAGE FUNCTIONS

4.2.1 Sensors of Invasion

As described in Chapter 2, macrophages possess both toll-like receptors (TLRs) and nucleotide-binding oligomerization domain–like receptors and so can detect invading bacteria and viruses. They respond by producing cytokines, the most important of which are interleukin-1 (IL-1), IL-12, IL-23, and tumor necrosis factor-α (TNF-α) (Figure 4-1).

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FIGURE 4-1 The cytokines secreted by macrophages and their functions.

MACROPHAGE

Interleukin-1
Co-stimulator of Th2 cells
Stimulates acute-phase response

Tumor necrosis factor-α
Cytotoxic
Stimulates T cell growth
Stimulates acute-phase response
Triggers inflammation

Interleukin-6
Promotes B cell differentiation
Stimulates acute-phase response

Interleukin-12
Co-stimulator of Th1 cells

Interleukin-18
Promotes IFN-γ production by Th1 cells

4.2.2 Phagocytosis

Monocytes bind to vascular endothelial cells in a manner similar to neutrophils. Thus cell rolling is triggered by selectin binding, and the cells are brought to a gradual halt by monocyte integrins binding ligands on blood vessel walls. Using b2 integrins, the monocytes bind to endothelial cell intercellular adhesion molecule-1 and emigrate through the vessel walls. Within tissues these cells are called macrophages. Several hours after neutrophils have entered an inflammatory site, the macrophages begin to arrive.

Macrophages are attracted not only by bacterial products and complement components such as C5a but also by alarmins released from damaged cells and tissues. Defensins and other peptides from neutrophils attract monocytes and macrophages. Activated neutrophils and endothelial cells produce monocyte chemoattractant protein-1 (CCL2) under the influence of IL-6. Neutrophils are thus the martyrs of the immune system: They reach and attack foreign material first, and in dying they attract macrophages to the site of invasion. Phagocytosis by macrophages is similar to the process in neutrophils. Macrophages destroy bacteria by both oxidative and nonoxidative mechanisms. In contrast to neutrophils, however, macrophages can undertake sustained, repeated phagocytic activity. In addition, macrophages secrete collagenases and elastases that destroy connective tissue. They also release plasminogen activator that generates plasmin, another potent protease. Thus macrophages can "soften up" the local connective tissue matrix and so permit more effective penetration of the damaged tissue.

Macrophages can phagocytose both apoptotic neutrophils and their granules. The contents of these neutrophil granules are not always destroyed but may be carried to endosomes, where they can inhibit the growth of bacteria such as Mycobacteria. Thus neutrophils can enhance the effectiveness of macrophages in host defense.

4.2.2.1

Generation of Nitric Oxide

In some mammals, especially rodents, cattle, sheep, and horses (but not in humans, pigs, goats, or rabbits), microbial products trigger macrophages to synthesize inducible nitric oxide synthase (NOS2). This enzyme uses NADPH and oxygen to act on L-arginine to produce large amounts of nitric oxide (nitrogen monoxide, NO) and citrulline ([Figure 4-2]). Although nitric oxide itself is not highly toxic, it can react with superoxide anion to produce highly reactive and toxic oxidants such as peroxynitrite and nitrogen dioxide radical.

$$NO + O_2^- \rightarrow OONO^- \rightarrow HOONO \rightarrow OH + NO_2^-$$
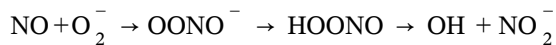
Nitric oxide   Peroxynitrite anion   Nitrogen dioxide radical

Not all macrophages generate nitric oxide. Those that do are called M1 cells, and their primary function is host defense. The sustained production of NO permits M1 macrophages to kill bacteria, fungi, protozoa, some helminths, and tumor cells very efficiently. Nitric oxide binds to metal-containing enzymes such as ribonucleotide reductase and impedes DNA synthesis. It also blocks mitochondrial heme-containing respiratory enzymes.

A second population of macrophages, called M2 cells, converts arginine to ornithine using the enzyme arginase and does not produce NO. These two macrophage populations play different roles in defending the body. M1 cells defend against microbial invaders and

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FIGURE 4-2 The two pathways of arginine metabolism in macrophages. The production of nitric oxide *(NO)* through the use of nitric oxide synthase 2 is a major antimicrobial pathway and the key feature of M1 macrophages. The use of arginase to produce ornithine, however, reduces the antimicrobial activities of M2 cells.

$$2NO + O_2 \longrightarrow 2NO_2$$

$$2NO_2 \rightleftharpoons N_2O_4 \longrightarrow NO_2^- + NO_3^-$$
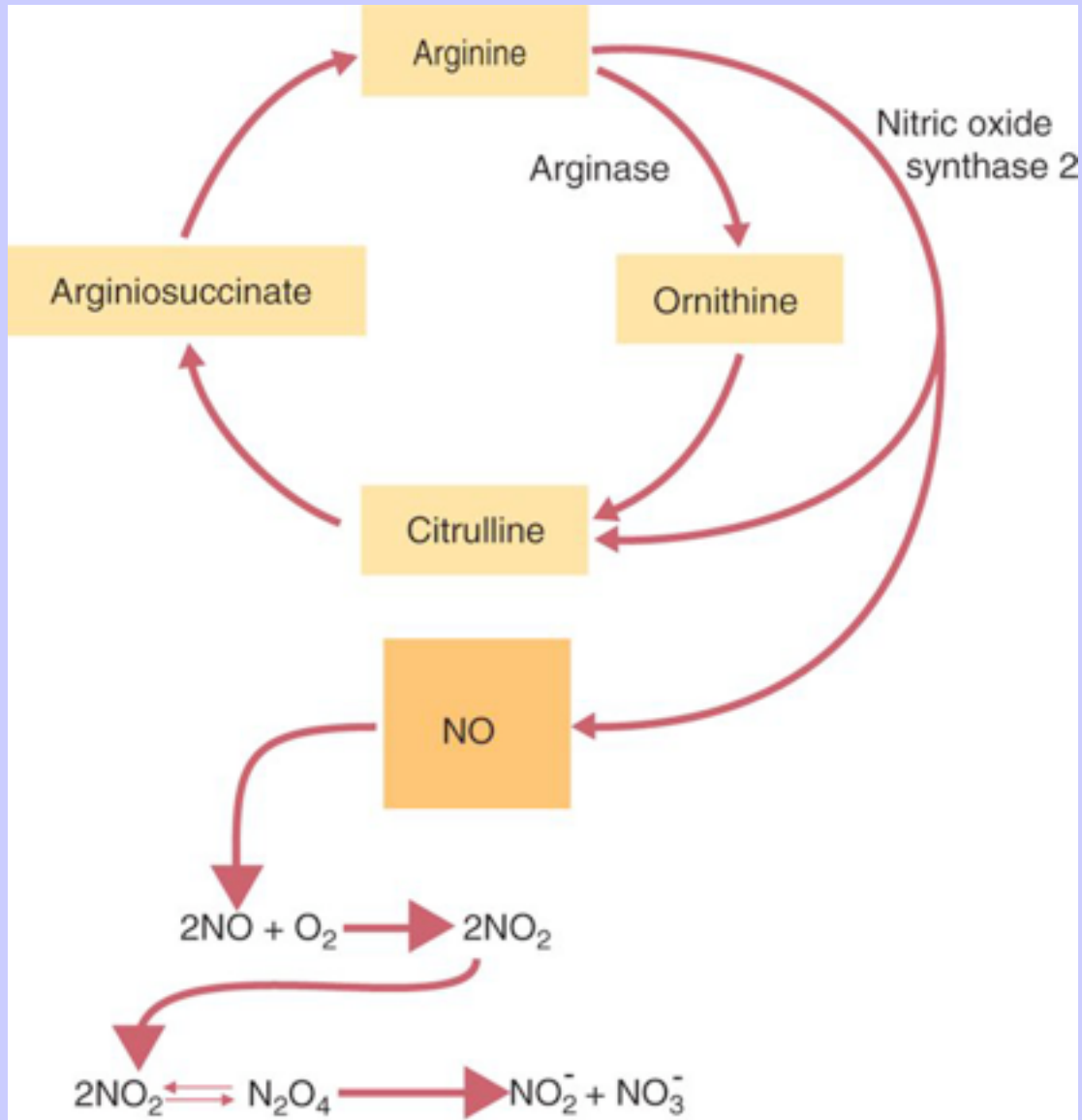
produce proinflammatory cytokines. M2 cells have opposite effects: They reduce inflammation and produce cytokines that suppress immune responses. M1 cells are produced early in the inflammatory process when inflammation is required. M2 cells, on the other hand, appear late in the process when healing is required. M2 cells thus promote blood vessel formation, tissue remodeling, and tissue repair.

4.2.2.1.1

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Box 4-1 Genes That Control Innate Immunity

Innate resistance to mycobacteria *Brucella, Leishmania,* and *Salmonella enterica typhimurium* is controlled by a gene called natural resistance-associated macrophage protein (Nramp), which has been identified in humans, dogs, mice, sheep, bison, red deer, cattle, and chickens. Nramp codes for an ion transporter protein in macrophages called natural resistance-associated transporter protein (Nramp1). After phagocytosis, Nramp1 is acquired by the phagosomal membrane. It then acts to pump divalent metals out of the phagosome and so inhibits the growth of intracellular parasites by depriving them of metal ions. Cattle with the resistant allele effectively activate their macrophages and so control the in vitro growth of *Brucella abortus*. The difference between the resistant and susceptible alleles appears to be associated with a single nucleotide substitution in the Nramp gene.

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4.2.3 Activation

Although macrophages are effective phagocytes, their activities can be enhanced by innate mechanisms. Activation triggers include the ligands for TLRs such as lipopolysaccharides, CpG DNA, microbial carbohydrates, and heat shock proteins as well as alarmins. Different levels of activation are recognized, depending on the triggering agent: some bacteria, such as *Mycobacterium tuberculosis,* are better able to activate macrophages than others. Thus when macrophages first move into inflamed tissues, they produce more lysosomal enzymes, increase phagocytic activity, increase the expression of antibody and complement receptors, and secrete more proteases ([Figure 4-3](#)). The cytokines produced by these M1 macrophages, especially TNF-α and IL-12, activate a population of lymphocytes called natural killer (NK) cells. The NK cells in turn secrete the cytokine interferon-γ (IFN-γ), which activates macrophages still further. IFN-γ upregulates many different genes, especially the gene for inducible nitric oxide synthase (NOS2). Thus the NOS2 gene can be upregulated 400-fold by a combination of IFN-γ and mycobacteria. As a result of increased NO production, M1 cells become very potent killers of bacteria ([Box 4-1](#)).

4.2.4 Receptors

Macrophages have many surface receptors, which may differ between subpopulations ([Figure 4-4](#)). In addition to the TLRs, they also possess receptors for antibodies. For example, CD64 is a high-affinity antibody receptor expressed on macrophages and to a lesser extent on neutrophils. Like other antibody receptors, CD64 binds the Fc region of antibody molecules and so is called an Fc receptor (FcγRI). Its expression is enhanced by IFN-γ–induced activation. Human macrophages also carry two low-affinity antibody receptors, CD32 (FcγRII) and CD16 (FcγRIII). Cattle macrophages have a unique Fc receptor called Fcg2R, which can bind particles coated with a specific type of antibody called IgG2.

Macrophages also have receptors for complement components. They include CD35 (CR1), the major receptor for C3b, and the integrin CD11b/CD18, which is also a receptor for fragments of C3b. These receptors permit macrophages to bind organisms coated with C3b.

The integrins described in the previous chapter are responsible for binding macrophages to other cells, to

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FIGURE 4-3 The progressive activation of macrophages can involve three pathways. Thus macrophages can become classically activated M1 cells by exposure to microbial products and/or to Th1 cytokines such as interferon-γ (*IFN*-γ). Alternatively they may undergo "alternative activation" on exposure to Th2 cytokines and so become M2 cells.
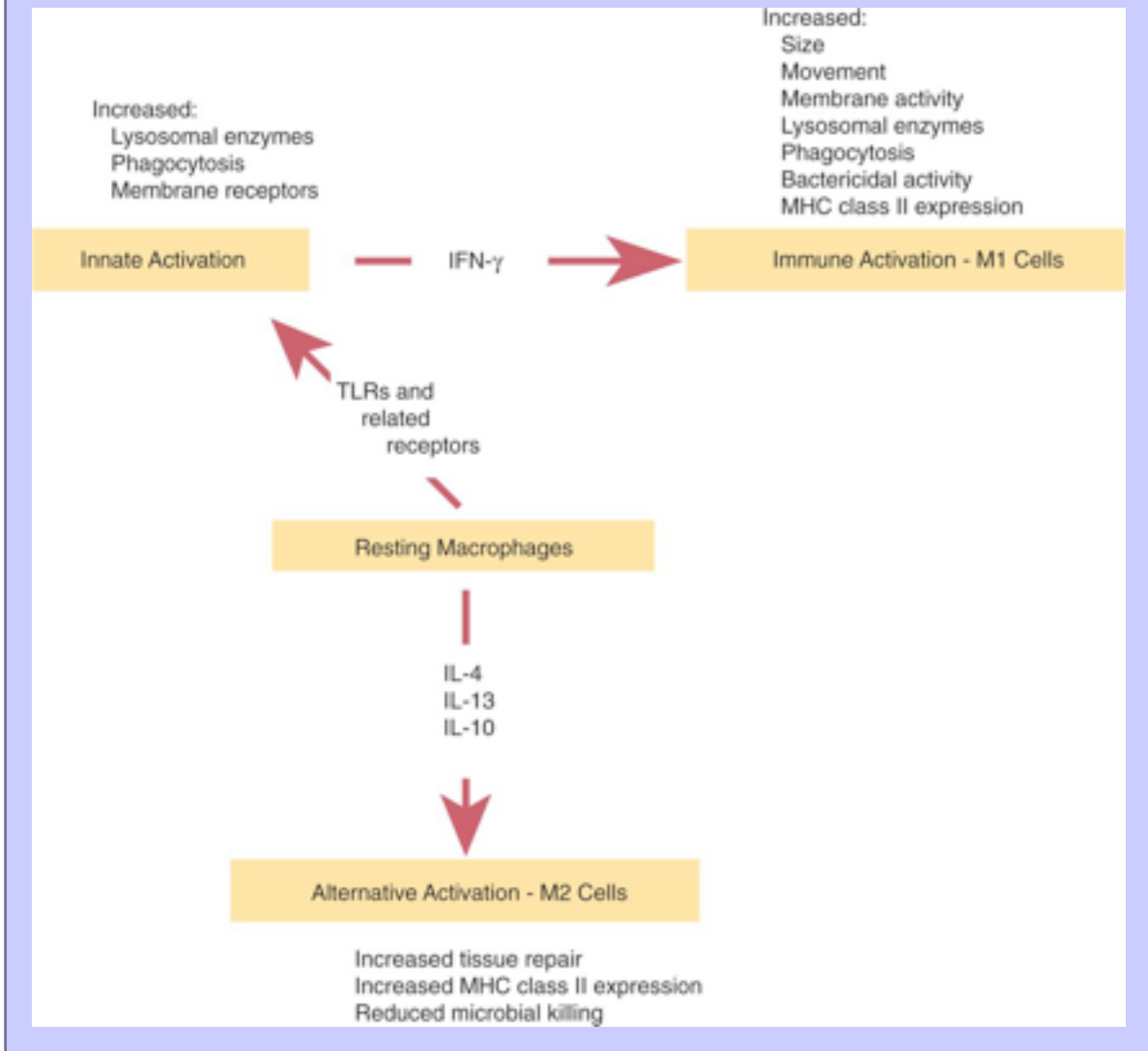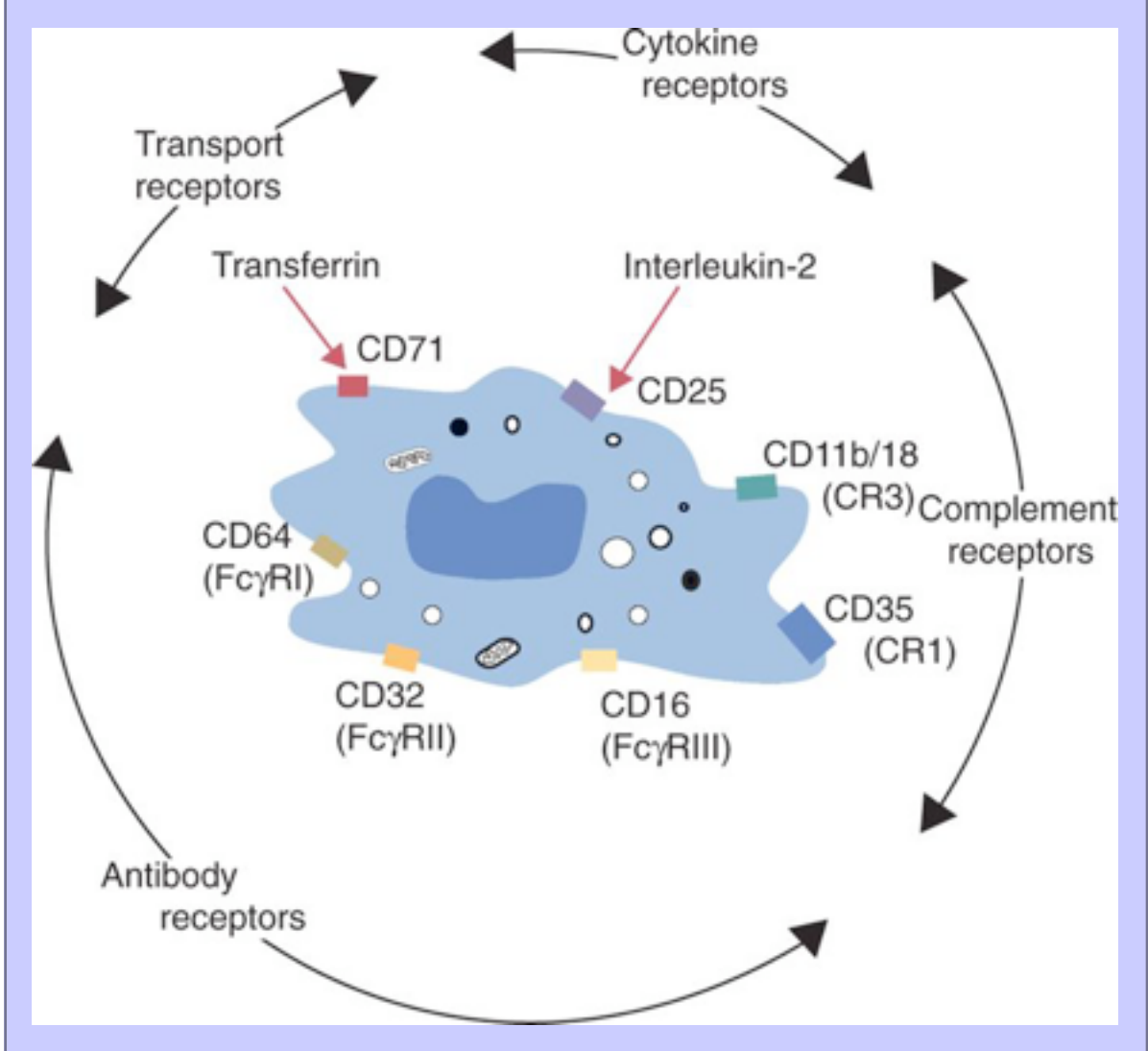
Increased:
Lysosomal enzymes
Phagocytosis
Membrane receptors

Increased:
Size
Movement
Membrane activity
Lysosomal enzymes
Phagocytosis
Bactericidal activity
MHC class II expression

Innate Activation — IFN-γ → Immune Activation - M1 Cells

TLRs and related receptors

Resting Macrophages

IL-4
IL-13
IL-10

Alternative Activation - M2 Cells

Increased tissue repair
Increased MHC class II expression
Reduced microbial killing

FIGURE 4-4 Some of the major surface receptors ex-pressed on macrophages and their functions.

connective tissue molecules such as collagen and fibronectin, and to some complement components. Macrophages also have mannose-binding receptors (CD206) that can bind to mannose or fucose in the capsule or lipopolysaccharide of invading bacteria and so permit macrophages to bind and ingest non-opsonized bacteria.

Another important macrophage receptor is CD40. This glycoprotein is used to communicate with lymphocytes. Its ligand is called CD40 ligand (CD40L or CD154) and is found on T cells. Macrophages also receive activation signals via CD40.

4.3 THE FATE OF FOREIGN MATERIAL

Macrophages are located throughout the body and hence can capture invaders entering by many different routes. For example, if bacteria are injected intravenously, they are rapidly removed from the blood. Their precise fate depends on the species involved. In

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FIGURE 4-5 The different routes by which bacteria are cleared from the bloodstream in the dog and cat. Dogs mainly use Kupffer cells in the liver. Cats mainly employ pulmonary intravascular macrophages.
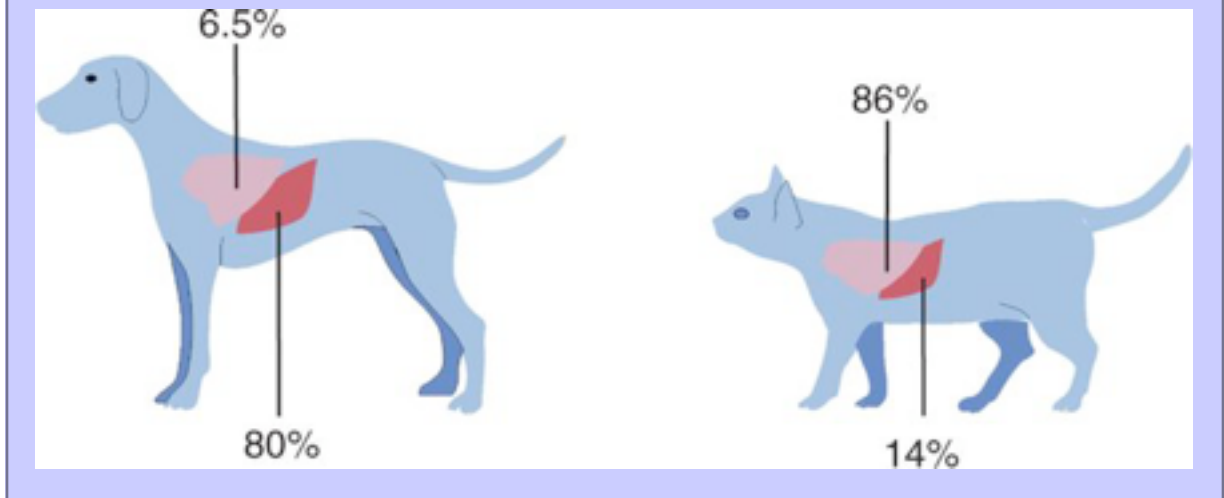
FIGURE 4-6 An intravascular macrophage *(M)* from the lung of a 7-day-old pig. The cell has numerous pseudopods, electron-dense siderosomes, phagosomes, and lipid droplets. It is closely attached to the thick portion of the air-blood tissue barrier that contains fibroblasts *(F)* and a pericyte *(P)* between basal laminae of the capillary endothelium *(E)* and the alveolar epithelium. At sites of close adherence, intercellular junctions with subplasmalemmal densities are seen (arrow). Bar = 2 μm (×8000). (From Winkler GC, Cheville NF: *Microvasc Res* 33:224-232, 1987.)
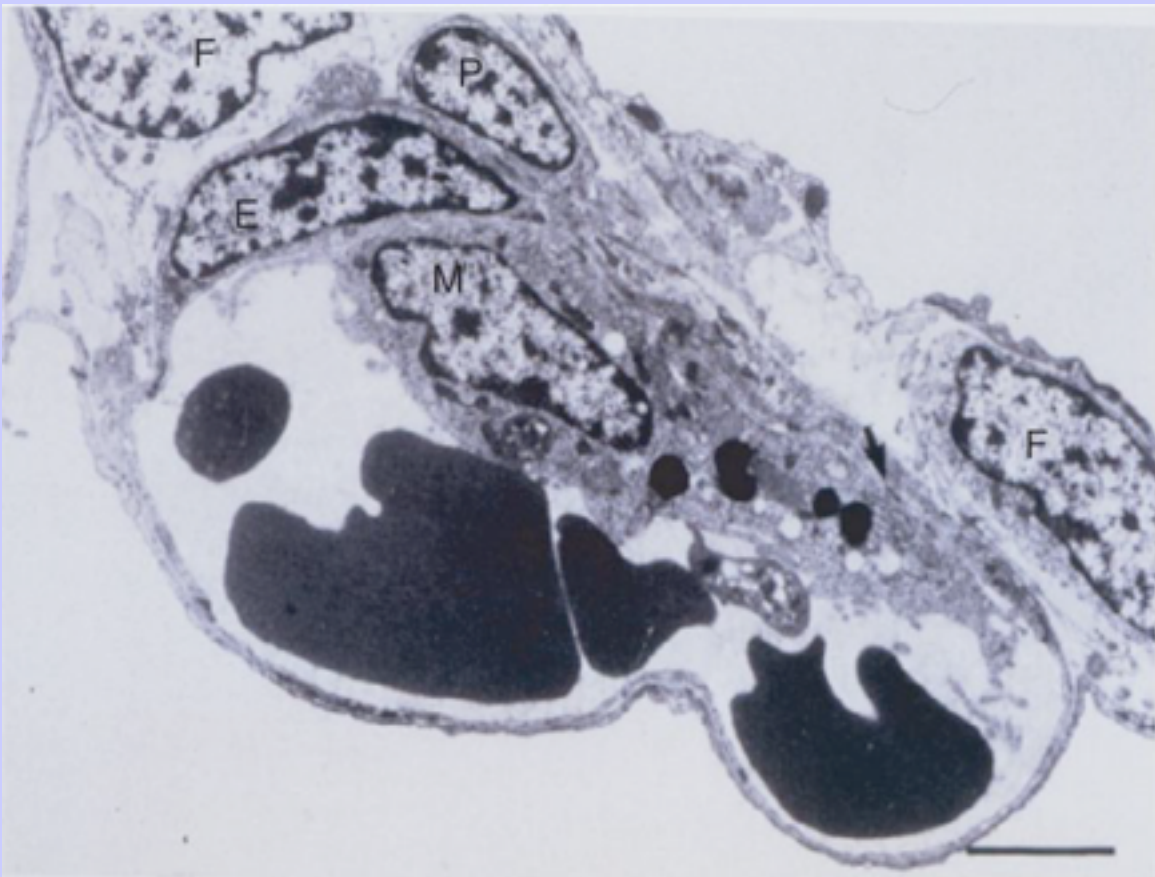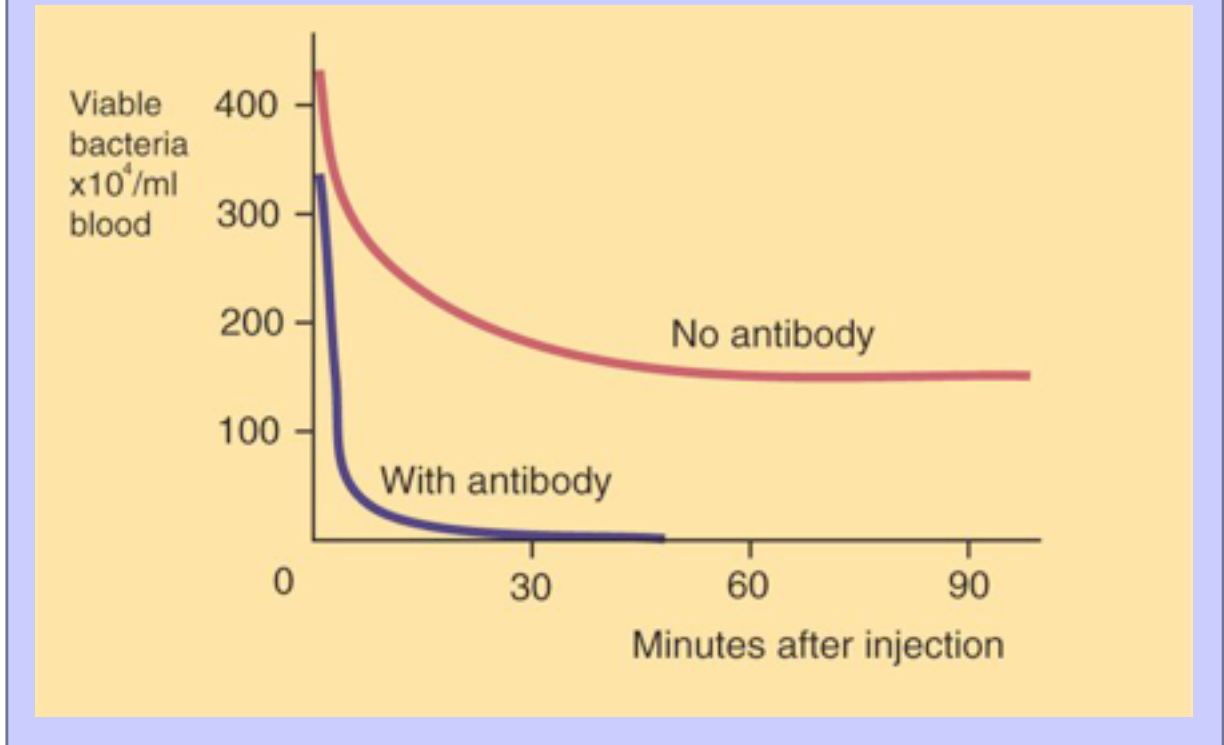
FIGURE 4-7 The clearance of bacteria from the blood (in this case *Escherichia coli* from piglets). In the absence of antibodies, bacteria are slowly and incompletely removed.

dogs, rodents, and humans, foreign particles are predominantly (80% to 90%) trapped and removed in the liver. Particles such as bacteria are removed by the macrophages (Kupffer cells) that line the sinusoids of the liver. The process occurs in two stages. Bacteria are first phagocytosed by neutrophils. These neutrophils are then ingested and destroyed by the Kupffer cells. These processes thus resemble acute inflammation, where neutrophils are primarily responsible for destruction of invaders, whereas macrophages are responsible for preventing damage caused by dying neutrophils (Table 4-1). In ruminants, pigs, horses, and cats, particles are mainly removed from the bloodstream by pulmonary intravascular macrophages (Figure 4-5). These macrophages line the endothelium of lung capillaries (Figure 4-6).

Species	Localization (%)	
	Lung	Liver/Spleen
Calf	93	6
Sheep	94	6
Dog	6.5	80
Cat	86	14
Rabbit	0.6	83
Guinea pig	1.5	82
Rat	0.5	97
Mouse	1.0	94

Table 4-1 Sites of Clearance of Particles from the Blood in Domestic Mammals

Selected data from Winkler GC: *Am J Anat* 181:223, 1988; and Chitko-McKown CG, Blecha F: *Ann Rech Vet* 23:201–214, 1992.

In species in which hepatic clearance is important, large viruses or bacteria may be cleared completely by a single passage through the liver (Figure 4-7). The spleen is a more effective filter than the liver, but since it is much smaller, it traps much less material. There are also differences in the type of particle removed by the liver and spleen. Splenic macrophages have antibody receptors (CD64) so that particles opsonized with antibody are preferentially removed in the spleen. In contrast, phagocytic cells in the liver express CD35, a receptor for C3, the third component of complement, so that particles opsonized by C3 are preferentially removed in the liver. The clearance of particles from the blood is regulated by opsonins such as fibronectin or mannose-binding lectin. If an animal is injected intravenously with a very large dose of colloidal carbon, these opsonins will be temporarily depleted and other particles (such as bacteria) will not be removed from the bloodstream. In this situation the mononuclear-phagocytic system is said to be "blockaded."

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Removal of organisms from the blood is greatly enhanced if they are opsonized by specific antibodies. If antibodies are absent or the bacteria possess an antiphagocytic polysaccharide capsule, the rate of clearance is decreased. Some molecules, such as bacterial endotoxins, estrogens, and simple lipids, stimulate macrophage activity and therefore increase the rate of bacterial clearance. Steroids and drugs that depress macrophage activity depress the clearance rate.

4.3.1
Soluble Proteins Given Intravenously

Unless carefully treated, protein molecules in solution tend to aggregate spontaneously. If a protein solution is injected intravenously, neutrophils, monocytes, and macrophages rapidly remove these protein aggregates. The unaggregated protein remains in solution and is distributed evenly through the animal's blood. Small proteins (less than 60 kDa) also spread throughout the extravascular tissue fluids. Once distributed, the protein is catabolized, resulting in a slow but progressive decline in its concentration. Within a few days, however, the animal mounts an immune response. Antibodies combine with the foreign antigen. Phagocytic cells remove these antigen-antibody complexes from the blood, and the protein is rapidly eliminated (Figure 4-8).

This triphasic clearance pattern of distribution, catabolism, and immune elimination may be modified under certain circumstances. For example, if the animal has not been previously exposed to an antigen, it takes between 5 and 10 days before immune elimination occurs. If, on the other hand, the animal has been primed by prior exposure to the antigen, a secondary immune response will occur in 2 to 3 days, and the stage of progressive catabolism will therefore be short. If antibodies are present at the time of antigen administration, immune elimination is immediate, and no catabolic phase is seen. If the injected material is not antigenic, or if an immune response does not occur, catabolism will continue until all the material is eliminated.

## 4.3.2 Fate of Material Administered by Other Routes

When foreign material is injected into a tissue, some damage and inflammation are bound to occur and alarmins are released. As a result, neutrophils and macrophages migrate toward the injection site and phagocytose the injected material. Some will, however, be captured by dendritic cells. The material taken up by macrophages and dendritic cells is processed and used to trigger acquired immunity. Antibodies and complement (see Chapter 5) interact with the antigenic material, generating chemotactic factors that attract still more phagocytic cells, thus hastening its final elimination. In the skin, a web of antigen-trapping dendritic cells called Langerhans cells may trap foreign molecules and present them directly to lymphocytes. For this reason intradermal injection of antigen may be most effective in stimulating an immune response.

Soluble material injected into a tissue is redistributed by the flow of tissue fluid through the lymphatic system. It eventually reaches the bloodstream, so its final fate is similar to intravenously injected material. Any aggregated material present is phagocytosed by neutrophils or tissue macrophages or by the macrophages and dendritic cells of lymph nodes through which the tissue fluid flows.

4.3.2.1 Digestive Tract

Digestive enzymes normally break molecules passing through the intestine into small fragments. However,

FIGURE 4-8 The clearance of a soluble antigen from the bloodstream. Note the three phases of this clearance.

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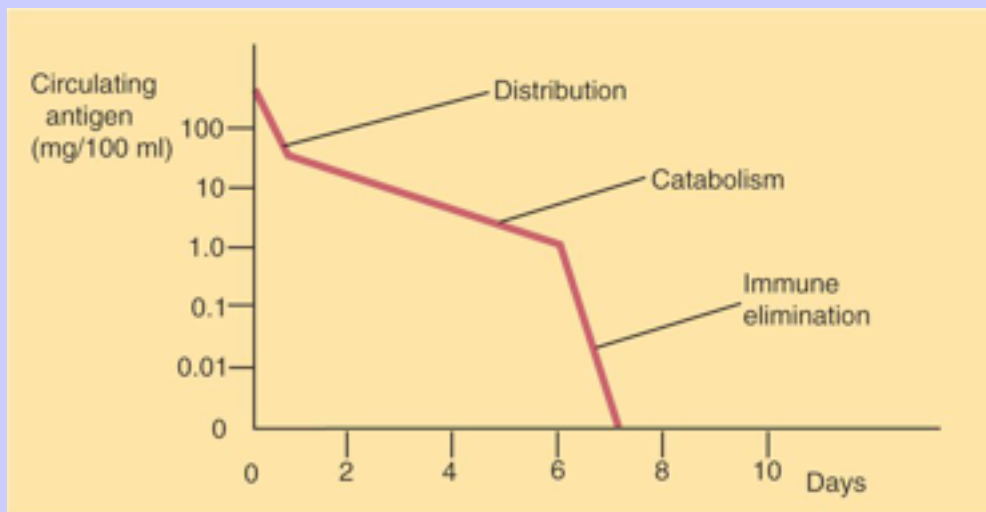
some molecules may remain intact and pass through the intestinal epithelium. Bacterial polysaccharides and molecules that associate with lipids are especially effective in this respect, since they are absorbed in chylomicrons. Particles that enter the blood from the intestine are removed by macrophages in the liver, whereas those particles entering the intestinal lymphatics are trapped in the mesenteric lymph nodes.

4.3.2.2

Respiratory Tract

The fate of inhaled particles depends on their size. Large particles (greater than 5 μm diameter) stick to the mucous layer that covers the respiratory epithelium from the trachea to the terminal bronchioles (see Chapter 19, Figure 19-3). These particles are then removed by the flow of mucus toward the pharynx or by coughing. Very small particles that reach the lung alveoli are ingested by alveolar macrophages, which carry them back to the bronchoalveolar junction; from there they are also removed by the flow of mucus. Nevertheless, some material may be absorbed from the alveoli. Small particles absorbed in this way are cleared to the draining lymph nodes, whereas soluble molecules enter the bloodstream and are distributed throughout the body. When large quantities of particles are inhaled, as occurs in workers exposed to industrial dusts or in cigarette smokers, the alveolar macrophage system may be "blockaded" and the lungs made more susceptible to microbial invasion.

4.4

RECOVERY FROM INFLAMMATION

Once invading organisms have been destroyed, the tissue response must switch from a killing process to a repair process. Thus as inflammation progresses, macrophages change their properties (Figure 4-9). They are first activated in the classical manner by TNF-α in order to kill ingested bacteria. However these M1 macrophages eventually convert to M2 cells and develop antiinflammatory properties. Thus the same cell can act in a proinflammatory manner at the beginning of an infection but switch to antiinflammatory activities once the infection is overcome.

Once they switch, M2 cells secrete SLP1, a serine protease inhibitor. This molecule inhibits the release of elastase and oxidants by TNF-α–stimulated neutrophils and inhibits the activity of the elastase. SLP1 also protects the antiinflammatory cytokine transforming growth factor-β (TGF-β) from breakdown, and TGF-β inhibits the release of TNF-α. Neutrophils also change during inflammation. Thus they secrete fragments of the TNF-α receptor that can bind and neutralize TNF-α. TNF-α stimulates macrophages to secrete IL-12, which in turn induces lymphocytes to secrete IFN-γ. The IFN-γ acts as a macrophage activator early in the inflammatory process, but later it becomes suppressive. Neutrophil-derived lipoxins suppress leukotriene synthesis.

Even in normal healthy animals, many cells die every day and must be promptly removed. Much of this task is the function of macrophages. A good example of this is the daily removal of enormous numbers of aged neutrophils. Macrophages, it appears, methodically "palpate" any neutrophils that they encounter. If the neutrophil is healthy, it quickly detaches from the macrophage. If, however, the neutrophil is dead or dying, the macrophage remains in contact and eats the neutrophil. This interaction operates through the adhesion protein CD31 (Figure 4-10). Thus CD31 on a neutrophil binds to CD31 on a macrophage. If the neutrophil is healthy, it sends a signal to

FIGURE 4-9 The role of M2 macrophages in tissue breakdown and tissue repair in wound healing.
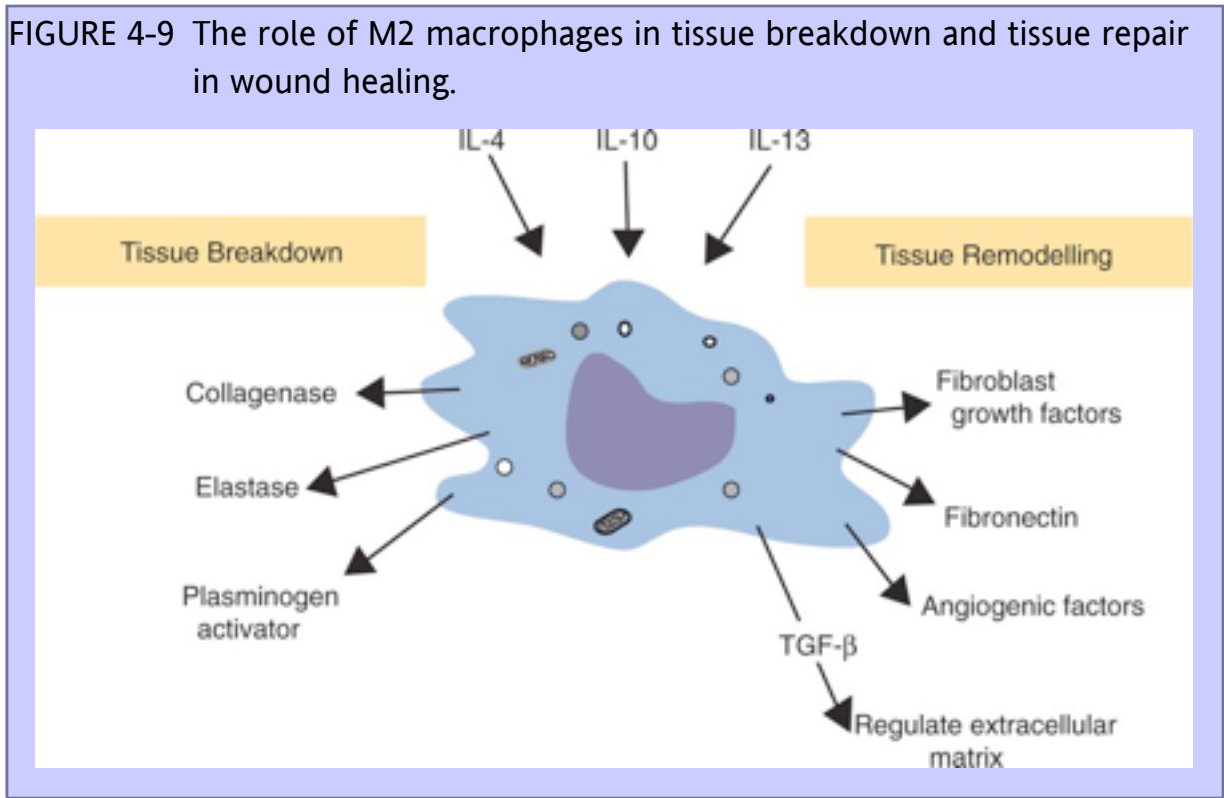
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FIGURE 4-10 The removal of apoptotic neutrophils. The reaction is initiated by interactions between CD31 on neutrophils and macrophages. If the neutrophil fails to reply when interrogated by a macrophage, it will be ingested and destroyed.
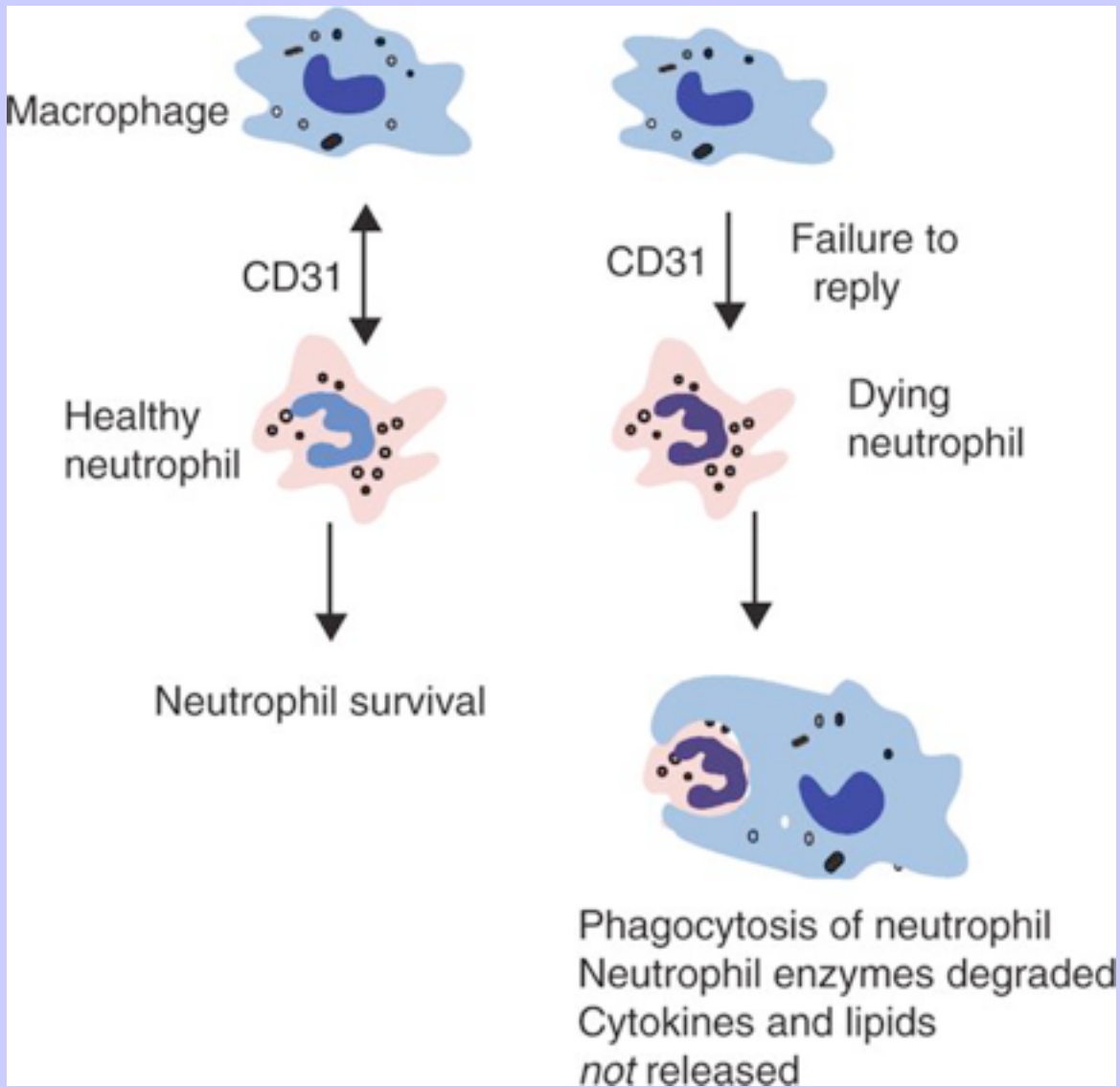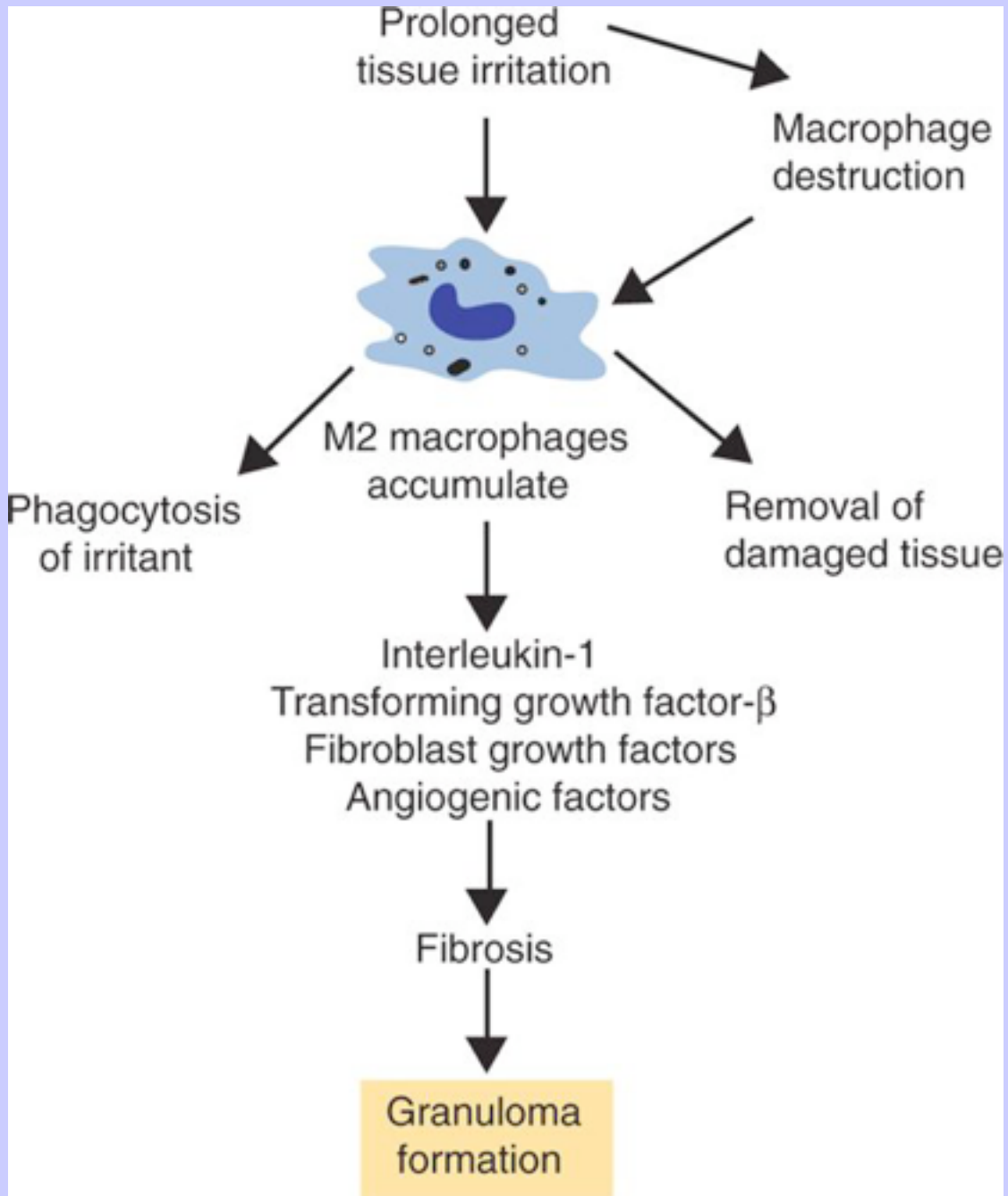
FIGURE 4-11 The pathogenesis of chronic inflammation. Macrophages undergoing prolonged stimulation may switch from an M1 to an M2 phenotype. M2 cells secrete cytokine mixtures that not only promote wound healing but also promote the "walling off" of persistent irritants by fibroblasts and extracellular matrix.

Prolonged tissue irritation

Macrophage destruction

M2 macrophages accumulate

Phagocytosis of irritant

Removal of damaged tissue

Interleukin-1
Transforming growth factor-β
Fibroblast growth factors
Angiogenic factors

Fibrosis

Granuloma formation

the macrophage, causing it to disengage. On the other hand, if the neutrophil fails to signal, it will be eaten. It is interesting to note that this failure in CD31 sig-naling occurs long before a neutrophil becomes so degraded that its contents can leak and cause damage. The macrophages that consume these neutrophils do not release cytokines or vasoactive lipids. Ingestion of apoptotic neutrophils does, however, cause the macrophages to secrete more TGF-β, which in turn promotes tissue repair. Phagocytosis is thus an efficient way of removing apoptotic neutrophils without causing additional tissue damage or inflammation.

By secreting IL-1β, macrophages attract and activate fibroblasts. The fibroblasts enter the damaged area and secrete collagen. Once sufficient collagen has been deposited, its synthesis stops. This collagen is then remodeled over several weeks or months as the area returns to normal. The reduced oxygen tension in dead tissues stimulates macrophages to secrete cytokines that promote the growth of new blood vessels. Once the oxygen tension is restored to normal, new blood vessel formation ceases.

The final result of this healing process depends on the effectiveness of the inflammatory response. If the cause is rapidly and completely removed, healing will follow uneventfully.

If tissue health is not restored, either because the invaders are not eliminated or because tissue repair is inadequate, inflammation may persist and become a damaging, chronic condition. Examples of persistent invaders include bacteria such as *M. tuberculosis,* fungi such as *Cryptococcus,* parasites such as liver fluke, or inorganic material such as asbestos crystals. Macrophages, fibroblasts, and lymphocytes may accumulate in large numbers around the persistent material over months or years. Because they resemble epithelium in histological sections, these accumulated macrophages are called epithelioid cells. Epithelioid cells may fuse and form multinucleated giant cells if they attempt to enclose particles too large to be ingested by a single macrophage. Epithelioid cells and giant cells are a prominent feature of tubercles, the persistent inflammatory lesions that develop in individuals suffering from tuberculosis (see Chapter 28).

In all these cases, the persistence of foreign material results in the continual arrival of new M2 macrophages, which continue to attract fibroblasts and stimulate the deposition of collagen. The chronic inflammatory lesion that develops around the foreign material is called a granuloma (Figure 4-11). Granulomas consist of granulation tissue —an accumulation of macrophages, lymphocytes, fibroblasts, loose connective tissue, and new blood vessels. The term *granulation* tissue is derived from the granular appearance of this tissue when cut. The "granules" are in fact new blood vessels.

If the persistent irritant is a nonantigenic "foreign body" (for example, silica, talc, or mineral oil), few neutrophils or lymphocytes will be attracted to the lesion. Epithelioid and giant cells, however, attempt to destroy the offending material. If the material is toxic for macrophages (as is asbestos), leaking enzymes may lead to progressive tissue damage, local fibrosis, and scarring.

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If the irritant is antigenic, the granuloma may contain many lymphocytes as well as macrophages, fibroblasts, and probably some neutrophils, eosinophils, and basophils (Figure 4-12). The chronically activated M2 cells within these granulomas secrete IL-1, which stimulates collagen deposition by fibroblasts and eventually "walls off" the lesion from the rest of the body. Granulomas are produced in response to bacteria such as the mycobacteria and *Brucella abortus* and parasites such as liver fluke and schistosomes. Chronic granulomas, whether due to immunological or foreign body reactions, are important since they may enlarge and destroy normal tissues. In liver fluke infestations, for example, death may result from the gradual replacement of normal liver cells by fibrous tissue formed as a result of the persistence of the parasites.

4.5 | SICKNESS BEHAVIOR

When an animal is invaded by microorganisms, a generalized response occurs—a response that we call sickness. The subjective feelings of sickness—malaise, lassitude, fatigue, loss of appetite, and muscle and joint pains—along with a fever, are components of innate immunity. They reflect a change in the body's priorities as it seeks to fight off invaders. Microbial molecules acting on the TLRs of phagocytic cells stimulate the production of IL-1β, IL-6, and TNF-α, which affect the brain (Figure 4-13). These cytokines signal to the brain by two routes. One route is direct through neurons that serve damaged tissue. IL-1 receptors are found on sensory neurons on the vagus nerve, and vagal sensory stimulation can thus trigger sickness responses in the brain. (IL-1β can make the vagus nerve excessively sensitive and so trigger nausea.) The second route involves circulating cytokines that either diffuse into the brain or are produced within the

FIGURE 4-12 A granulomatous inflammatory reaction around a degenerating tapeworm cyst in a bovine heart. The mass of cells around the central organism is a mixture of macrophages and fibroblasts serving to wall it off from the rest of the body (×250). (Courtesy Dr. John Edwards.)
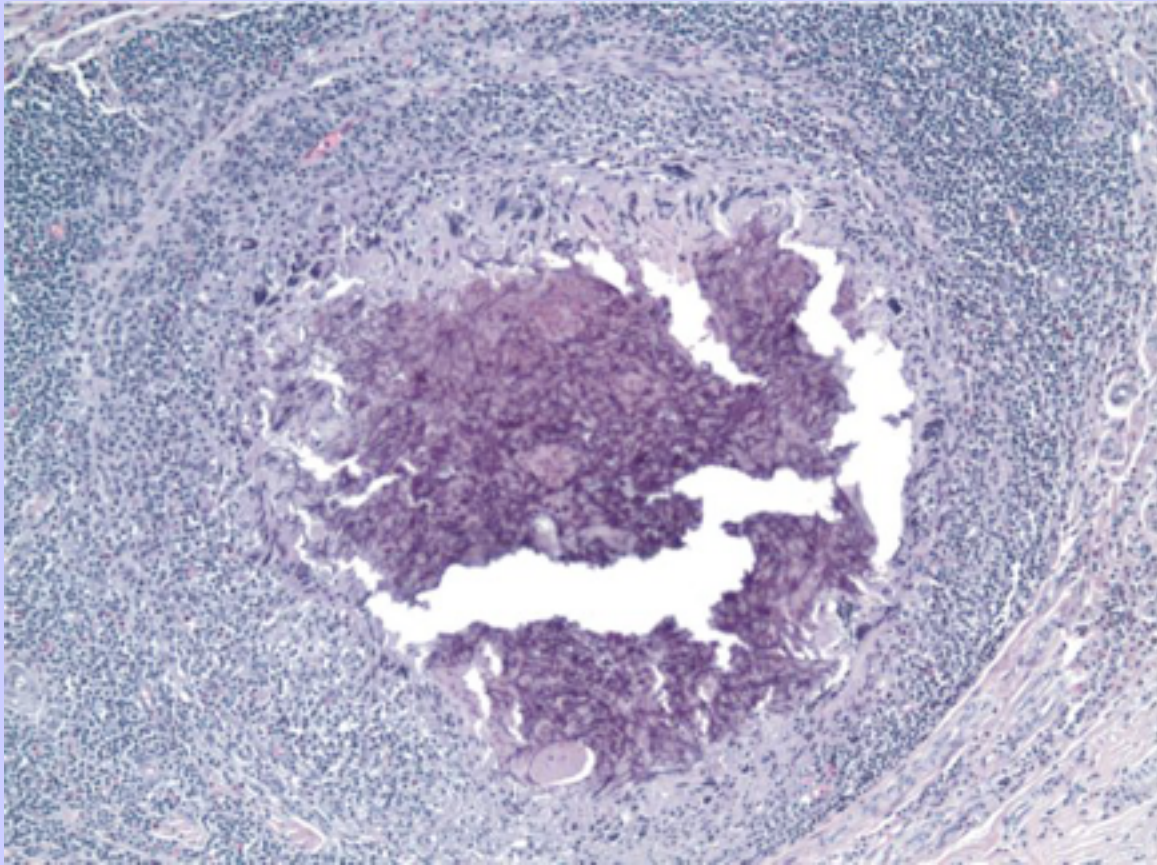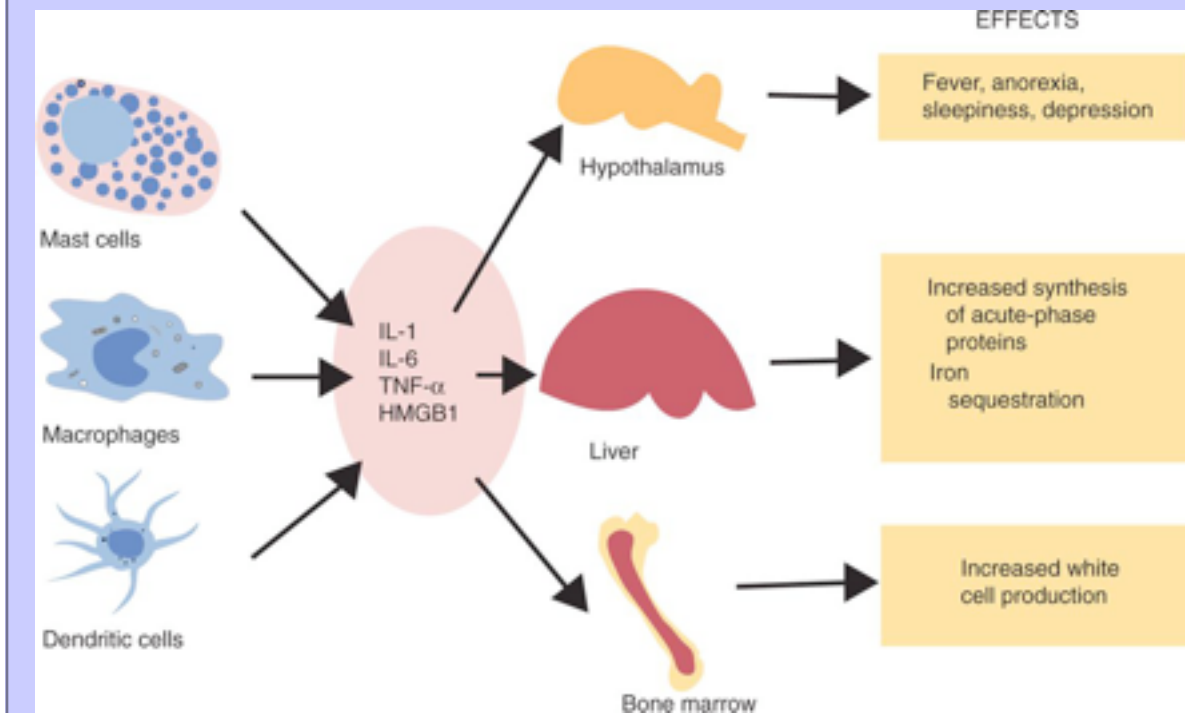
FIGURE 4-13 Sickness behavior is part of the response of the body to inflammatory stimuli. Multiple systemic effects are due to the four major cytokines secreted by sentinel cells, mast cells, macrophages, and dendritic cells. The major sickness-inducing cytokines are interleukin-1 *(IL-1)*, IL-6, tumor necrosis factor-α *(TNF-α)*, and high mobility group box protein-1 *(HMGB1)*.

brain. These cytokines act on the brain to modify behavior.

One of the most obvious features of the brain's response to infection is the development of a fever. IL-1, IL-6, and TNF-α all act on the brain to induce sleep, suppress appetite, and raise body temperature (except in mice, whose temperature drops). These cytokines induce prostaglandin production, which causes the body's thermostatic set-point to rise. In response, animals conserve heat by vasoconstriction and increase heat production by shivering, thus causing the body temperature to rise until it reaches the new set-point. This fever enhances some components of the immune responses. For example, elevated body temperatures cause dendritic cells to mature, enhance the circulation of lymphocytes, and promote the secretion of IL-2. Fever range temperatures greatly enhance the survival of T cells by inhibiting their apoptosis. The cytokines released during inflammation, especially IL-1, are also responsible for the reduction in social behavior seen in sickness; they promote the release of sleep-inducing molecules in the brain. Increased lethargy is commonly associated with a fever and may, by reducing the energy demands of an animal, increase the efficiency of defense and repair mechanisms. IL-1 also suppresses the hunger centers of the brain and so induces the loss of appetite associated with infections. The benefits of this are unclear, but it may permit the animal to be more selective about its food. If the anorexia persists, it can have an adverse effect on growth.

High mobility group box protein-1(HMGB1) is a potent sickness-inducing cytokine. Although IL-1, IL-6, and TNF-α have long been known to be mediators of septic shock and sickness behavior, it is now clear that these three molecules plus IFN-γ induce HMGB1 release from macrophages several hours after initiation of sickness. It enters secretory lysosomes and is released slowly from the cells. HMGB1 has been implicated in food aversion and weight loss by its actions on the hypothalamic-pituitary axis. It mediates endotoxin lethality, arthritis, and macrophage activation. It is likely that the inflammation induced by necrotic cells is caused by the release of HMGB1 from disrupted nuclei. HMGB1 is an excellent example of an alarmin.

4.5.1 Metabolic Changes

In addition to their effects on the nervous and immune systems, IL-1, IL-6, and TNF-α act on skeletal muscle to enhance protein catabolism and thus mobilize a pool of available amino acids. Although this eventually results in muscle wastage, the newly available amino acids are available for increased antibody synthesis. Other systemic responses include the development of a neutrophilia (elevated blood neutrophils) as a result of enhanced stem cell activity, weight loss due to muscle wasting and loss of adipose tissue, and the production of many new proteins (acute-phase proteins) that help fight infection.

Animals exposed to chronic, sublethal doses of TNF-α lose weight and become anemic and protein depleted. The weight loss occurs because TNF-α inhibits the synthesis of enzymes necessary for the uptake of lipids by preadipocytes and causes mature adipocytes to lose stored lipids. TNF-α is thus responsible for the weight loss seen in animals with cancer or chronic parasitic and bacterial diseases.
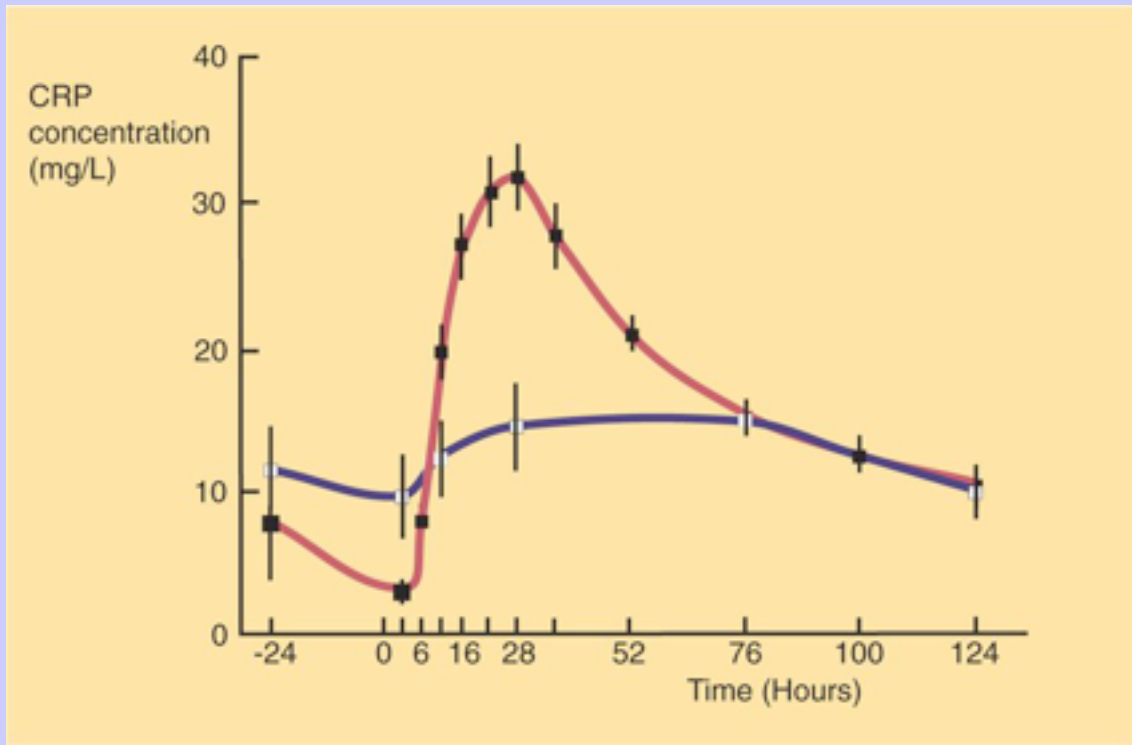
4.5.2 Acute-Phase Proteins

Under the influence of IL-1β, TNF-α, and especially IL-6, liver cells greatly increase their protein synthesis and secretion. This begins within a few hours of injury and subsides within 24 to 48 hours (Figure 4-14). Because this is associated with acute infections and inflammation, the newly produced proteins are called acute-phase proteins. Many of the acute-phase proteins are important components of the innate immune system. They include complement components, clotting molecules, protease inhibitors, and metal-binding proteins. Different mammals produce different acute-phase proteins (Figure 4-15).

C-reactive protein (CRP) is the major acute-phase protein in primates, rabbits, hamsters, and dogs and is important in pigs. CRP is a pentraxin and so has a pentameric structure with two faces. One face binds to phosphocholine, a common side chain found in all cell membranes and many bacteria and protozoa. The other face is responsible for binding to neutrophils through the antibody receptors FcγRI and FcγRIIa and to the complement component C1q. CRP can thus promote the phagocytosis and removal of damaged, dying, or dead cells as well as microorganisms. CRP can bind to bacterial polysaccharides and glycolipids and to healthy and damaged cells, where it activates C1q and the classical complement pathway. (Its name derives from its ability to bind and precipitate the C-polysaccharide of *Streptococcus pneumoniae*). CRP also has an antiinflammatory role since it inhibits neutrophil superoxide production and degranulation and blocks platelet aggregation. CRP may therefore promote healing by reducing damage and enhancing the repair of damaged tissue. The functions of CRP differ between species. For example, in cattle, the level of CRP rises twofold to fivefold in lactating cows.

Serum amyloid A (SAA) is the major acute-phase protein in cattle, cats, and horses and is also important in humans and dogs. Thus equine SAA concentrations rise several hundred–fold during noninfectious arthritis, while canine SAA concentrations increase up to twentyfold in bacterial disease. Since SAA protein

FIGURE 4-14 The rise in C-reactive protein levels in six dogs following anesthesia and surgery *(red line)* and in six dogs undergoing anesthesia alone *(blue line)*. (From Burton SA, Honor DJ, Mackenzie AL, et al: *Am J Vet Res* 55:615, 1994.)

is immunosuppressive, it probably regulates immune responses. SAA is a chemoattractant for neutrophils, monocytes, and T cells. SAA increases significantly in mastitic milk.

Serum amyloid P (SAP) is the major acute-phase protein in rodents. It is a pentraxin related to CRP. Like CRP, one face of the molecule can bind nuclear constituents such as DNA, chromatin, and histones as well as cell membrane phospholipids. The other face can also bind and activate C1q and thus activate the complement system.

Haptoglobin is a major acute-phase protein in ruminants, horses, and cats. It can rise from virtually undetectable levels in normal calves to as high as 1 mg/ml in calves with acute respiratory disease. Haptoglobin binds iron molecules and makes them unavailable to invading bacteria, thus inhibiting bacterial proliferation and invasion. Haptoglobin also binds free hemoglobin, thus preventing its oxidation of lipids and proteins. It is possible to identify animals with severe infections or inflammatory conditions by measuring serum haptoglobin levels. This may be of benefit in antemortem meat inspections by identifying those animals that are not fit to eat. Other iron-binding acute-phase proteins include transferrin (important in birds) and hemopexin.

Hepcidin is another iron-binding protein produced by hepatocytes under the influence of IL-6. Hepcidin suppresses intestinal iron absorption and macrophage iron release. As a result of the increase in hepcidin and haptoglobin, iron availability for red blood cell production drops and chronically infected animals become anemic —the anemia of infection.

Major acute-phase protein (MAP) is the major acute-phase protein in pigs and a substrate for the proteolytic enzyme kallikrein and so releases inflammatory peptides called kinins.

Other acute-phase proteins include lipopolysac-charide-binding protein (cattle); CD14 (humans and mice); collectins such as mannose-binding lectin and conglutinin (many species); ceruloplasmin and fibrinogen (sheep); and ceruloplasmin (pigs). Some serum protease inhibitors such as α_1-antitrypsin, α_1-antichymotrypsin, and α_2-macroglobulin are acute-phase proteins in many mammalian species. All of these may inhibit neutrophil proteases in sites of acute inflammation.

Some protein levels fall during acute inflammation. These are called "negative" acute-phase proteins. In the pig, for example, these include albumin, fetuin, transferrin, transthyretin, and apolipoprotein A-1.

The two cytokines IL-1 and IL-6 have quite different effects on the liver and, as a result, are used to classify the acute phase proteins into two types. Type 1 acute phase proteins are those that require both IL-6 and IL-1 for maximum synthesis. Examples of type 1 acute phase proteins include CRP, SAA, and alpha-1 acid glycoprotein. Type 2 acute phase proteins, in contrast, require only IL-6 for maximum production. Examples of type 2 acute phase proteins include fibrinogen, haptoglobin, and alpha-2 macroglobulin.

4.6 SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

In severe infections or after massive tissue damage, very large amounts of cytokines and oxidants may be produced, escape into the bloodstream, and cause

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FIGURE 4-15 Species differences in the major acute-phase proteins produced by the domestic mammals.
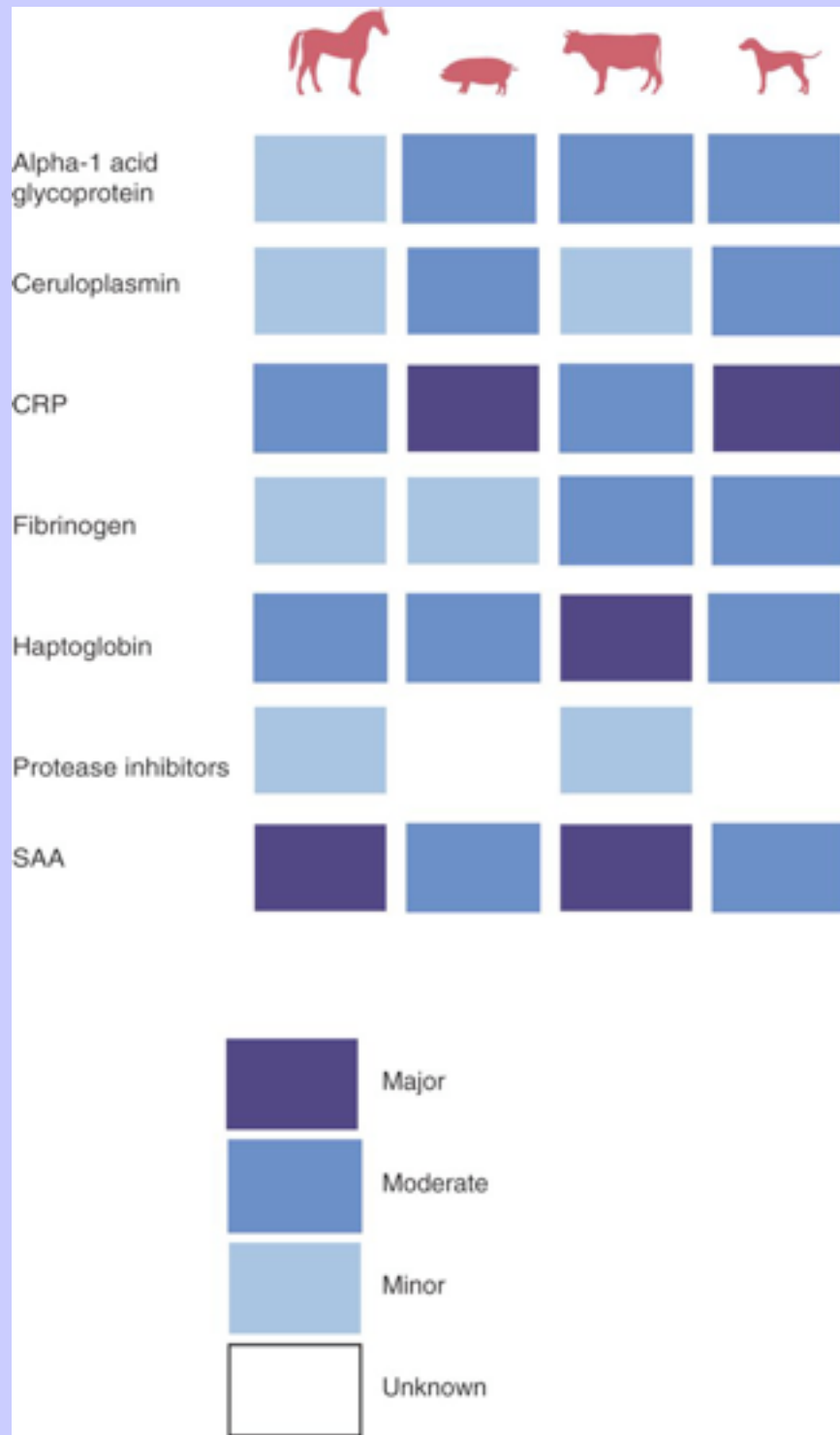
a lethal form of shock known as systemic inflam-matory response syndrome or, more simply, sepsis. Many different infectious diseases are characterized by the activation of large numbers of immune cells and the consequent production of large amounts of many different cytokines and inflammatory mediators within a short period of time. Since many cytokines are relatively toxic, this "cytokine storm" may cause severe toxicity, tissue damage, and even death. The most important of these include TNF-α, IFN-γ, IL-8, and IL-6. These cytokines may trigger the activation of additional T cells and the release of additional cytokines and therefore trigger a cytokine storm.

The most obvious of these cytokine storms is that resulting from tissue trauma, infections, or burns that give rise to septic shock. However, many important infections such as influenza, dengue, Gram-negative bacterial infections, filoviruses, and malaria may also trigger excessive cytokine release and death. Other diseases involving cytokine toxicity include graft-versus-host disease. Different triggers probably induce production of different cytokine mixtures at different sites so that the pathology of these diseases may be variable. Among the most important toxic effects is activation of endothelial cells leading to increased vascular permeability and intravasccular coagulation.

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4.6.1

Bacterial Septic Shock

Septic shock is the name given to the systemic inflammatory response syndrome caused by severe infections and associated with trauma, ischemia, and tissue injury. It accounts for about 9% of human deaths in the United States and is a correspondingly important cause of animal deaths. Animals or humans with mild infections develop the characteristic signs of sickness such as fevers, rigors, myalgia, depression, headache, and nausea as a result of cytokine release. Severe infections, however, may result in vastly excessive cytokine production that leads to severe acidosis, fever, lactate release in tissues, an uncontrollable drop in blood pressure, elevation of plasma catecholamines, and eventually to renal, hepatic, and lung injury and death. The procoagulant-anticoagulant balance is upset so that endothelial procoagulant activity is enhanced while many anticoagulant pathways are inhibited, leading to intravascular coagulation and capillary thrombosis ([Figure 4-16](#)).

All these effects are mediated by excessive triggering of TLRs leading to a massive and uncontrolled release of HMGB1 and other cytokines. TLRs 4 and 2 and HMGB1 receptors trigger a "cytokine storm," from endotoxin-stimulated macrophages. Other cytokines involved include TNF-α and IL-1β, with IFN-γ, IL-6, and CXCL8 (IL-8) in a supporting role. These cyto-kines in turn stimulate expression of NOS2 leading to an increase in serum nitric oxide and of cyclooxygenase-2, which in turn leads to prostaglandin and leuko-triene synthesis. The cytokines damage vascular endothelial cells, activating them so that procoagulant activity is enhanced, resulting in blood clotting. The nitric oxide causes vasodilation and a drop in blood pressure. The prostaglandins and leukotrienes cause increases in vascular permeability. The widespread damage to vascular endothelium eventually causes organ failure.

Multiple organ dysfunction syndrome is the end stage of severe septic shock. It is characterized by hypotension, insufficient tissue perfusion, uncontrollable bleeding, and organ failure caused by hypoxia, tissue acidosis, tissue necrosis, and severe local metabolic disturbances. The severe bleeding is due to disseminated intravascular coagulation.

The sensitivity of mammals to septic shock varies greatly. Species with pulmonary intravascular macrophages (cat, horse, sheep, and pig) tend to be more susceptible than dogs and rodents, which lack pulmonary intravascular macrophages and are thus relatively insusceptible to lung injury. It is of interest to note that in foals with sepsis, TLR4 gene expression is greatly increased and a poorer prognosis is associated with increased expression of IL-10.

4.6.2 Bacterial Toxic Shock

Some strains of *Staphylococcus aureus* produce enterotoxins that bind and stimulate T cell antigen receptors ([Figure 4-17](#)). These toxins may thus stimulate up to 20% of an animal's T cells, causing them to secrete enormous quantities of IL-2 and IFN-γ. These in turn stimulate production of TNF-α and IL-1β. This leads to the development of a fever, hypotension, collapse, skin lesions, and damage to the liver, kidney, and intestines with multiple organ dysfunctions called toxic shock syndrome. A similar syndrome has also been observed in some streptococcal infections. In these cases, streptococcal M-protein binds to fibrinogen. The M-protein-fibrinogen complexes bind to endothelial cell integrins and trigger a respiratory burst. This causes an increase in vascular permea-bility and hypercoagulability leading to toxic shock characterized by hypotension and disseminated intravascular coagulation.

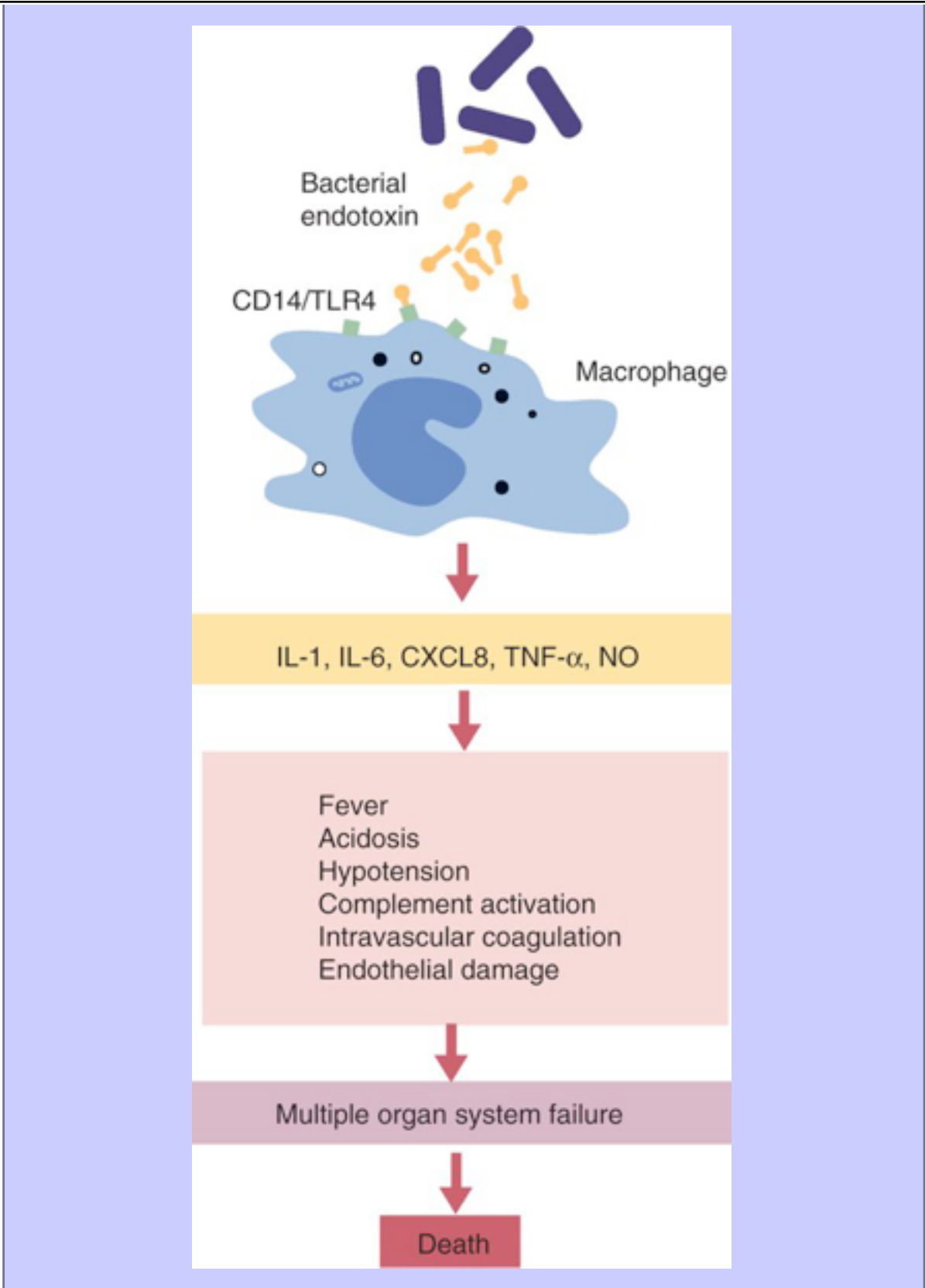FIGURE 4-16 The pathogenesis of the systemic inflammatory response syndrome.

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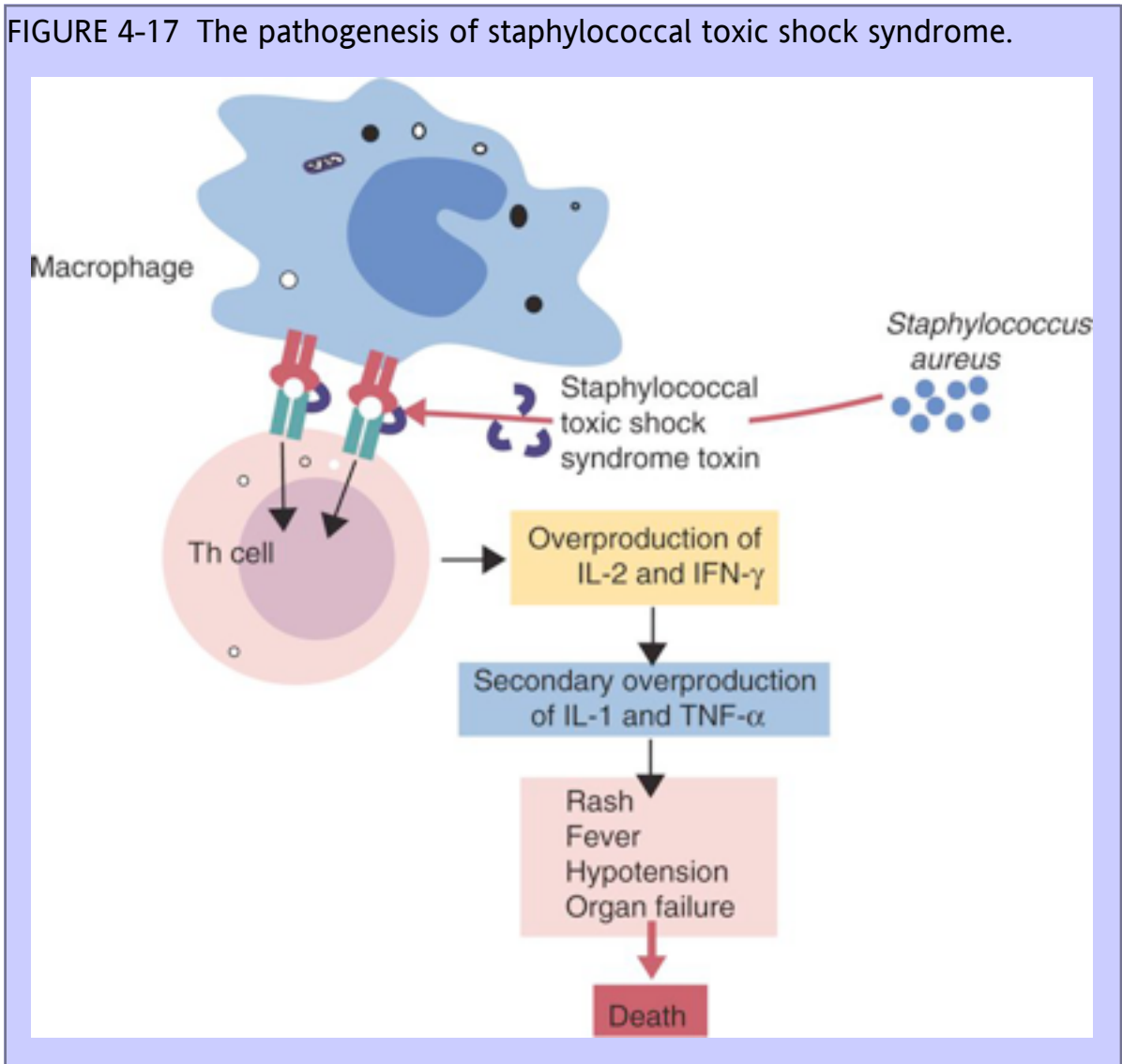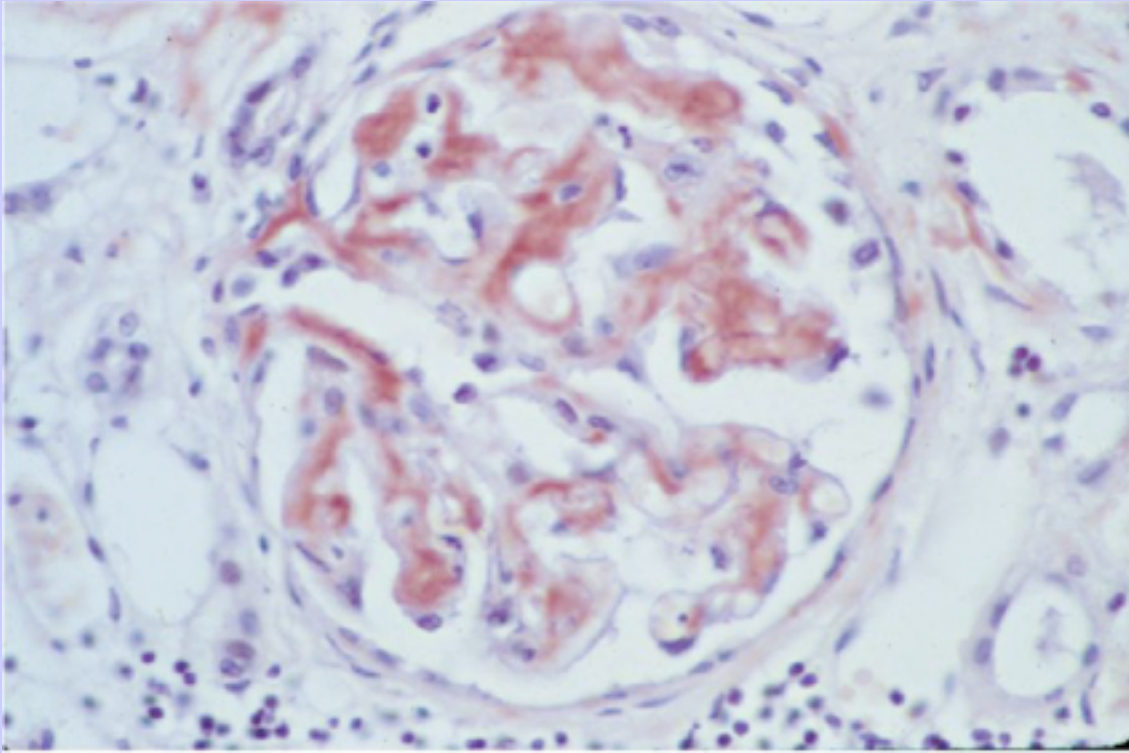FIGURE 4-17 The pathogenesis of staphylococcal toxic shock syndrome.

FIGURE 4-18 Secondary amyloid deposited in a glomerulus. The red dye (Congo red) specifically binds to amyloid fibrils (×400).

4.6.3 Graft-versus-Host Disease

Another syndrome characterized by excessive production of cytokines, especially TNF-α, is graft-versus-host disease. In this disease, described in more detail in Chapter 29, grafted lymphocytes attack the tissues of the graft recipient. TNF-α from these cells causes mucosal destruction, leading to ulceration, diarrhea, and liver destruction.

4.7 PROTEIN MISFOLDING DISEASES

Amyloidosis is the name given to the deposition of insoluble proteins in tissues. These deposits appear as amorphous, eosinophilic, hyaline proteins in cells and tissues (Figure 4-18). Amyloid is produced as a result of errors in the folding of newly formed protein chains. These misfolded chains eventually aggregate to form insoluble fibrils. Electron microscopy shows that amyloid proteins consist of protein fibrils formed by peptide chains cross-linked to form β-pleated sheets (Figure 4-19). This molecular conformation makes amyloid proteins extremely insoluble and almost totally resistant to proteases. Consequently, once deposited in cells or tissues, amyloid deposits are almost impossible to remove. Amyloid infiltration eventually leads to gradual cell loss, tissue destruction, and death. Amyloidosis may be systemic (involving multiple organs) or localized (involving only one organ).

Many different proteins can misfold and so form amyloid. For example, amyloidosis may develop when infections or inflammation cause a sharp rise in the concentration of the acute-phase protein SAA. A 76-residue proteolytic fragment of SAA can accumulate, misfold, aggregate, and be deposited in multiple organs. This material, one of the most common forms in domestic animals, is called reactive amyloid. Reactive amyloidosis is associated with chronic inflam-mation in diseases such as mastitis, osteomyelitis, abscesses, traumatic pericarditis, and tuberculosis

FIGURE 4-19 Amyloid fibrils. An electron micrograph showing bundles of paired amyloid fibrils deposited parallel to a cell membrane. (Courtesy Dr. E.C. Franklin. From Franklin EC: Adv Immunol 15:25, 1972.)
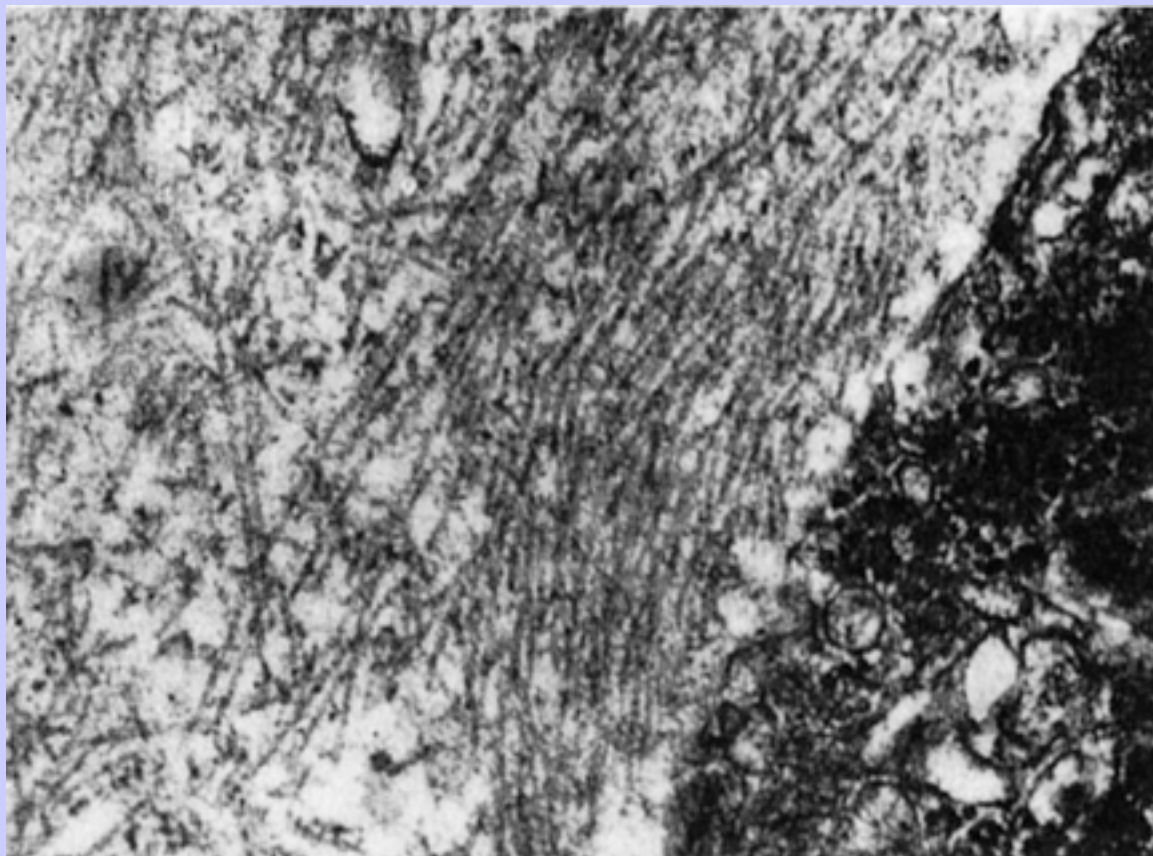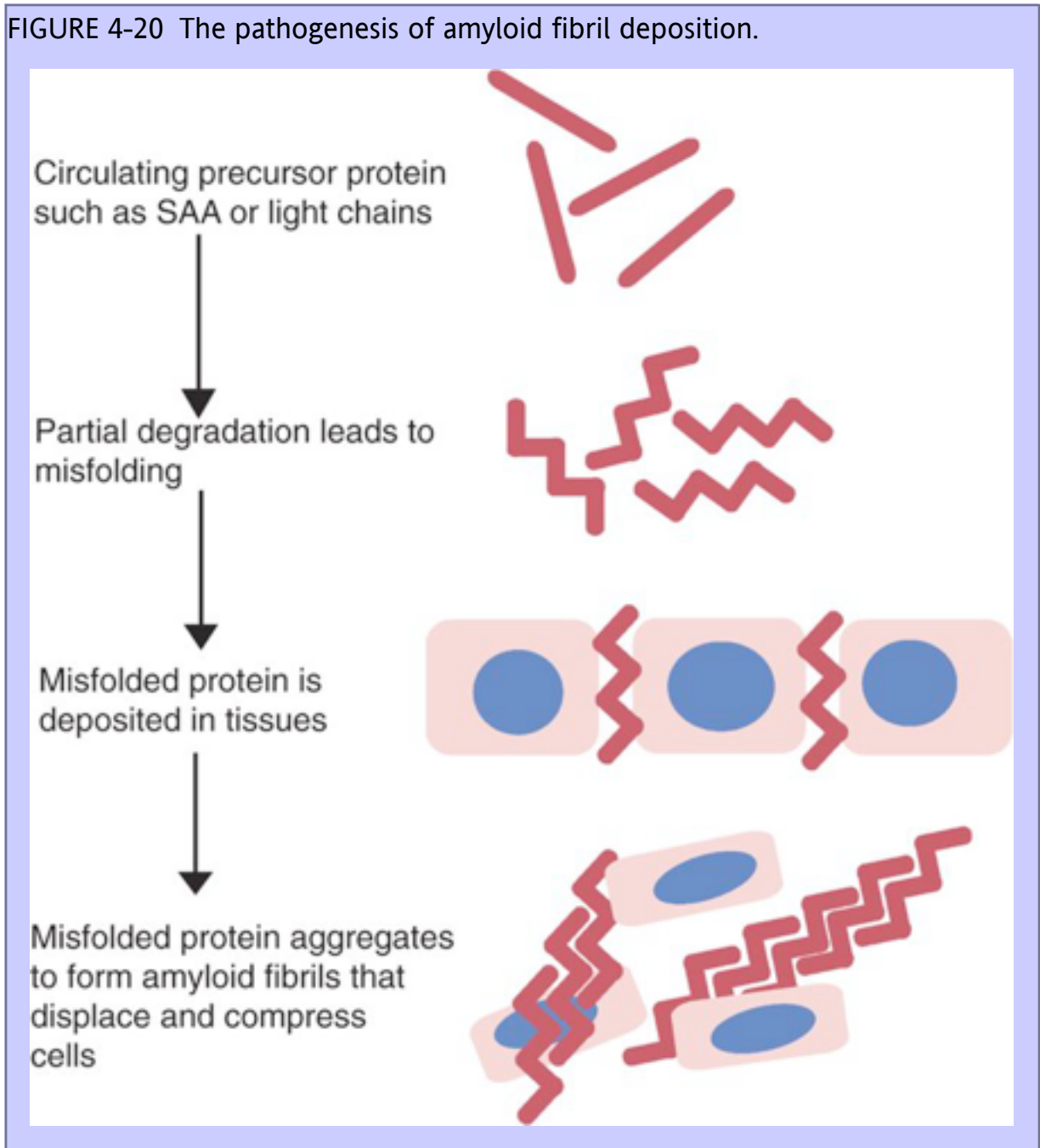
FIGURE 4-20 The pathogenesis of amyloid fibril deposition.

Circulating precursor protein such as SAA or light chains

Partial degradation leads to misfolding

Misfolded protein is deposited in tissues

Misfolded protein aggregates to form amyloid fibrils that displace and compress cells

(Figure 4-20). Reactive amyloidosis is a major cause of death in animals repeatedly immunized for commercial antiserum production. Familial amyloidosis of Shar-Pei dogs consists of reactive amyloid deposited following chronic immune-mediated arthritis.

Multiple myelomas are plasma cell tumors that secrete antibodies, especially antibody light chains (see Chapter 13). Their presence leads to the production of huge quantities of antibody light chains and their fragments. The misfolding of these light chains and their fragments results in the deposition of immunogenic amyloid (AL). Although AL amyloid is the most common form of amyloid in humans, it is very rare in domestic animals.

Several forms of localized amyloidosis are recognized in domestic animals. For example, old dogs may suffer from vascular amyloidosis, in which amyloid is deposited in the media of leptomeningeal and corti-cal arteries. An inherited form of amyloid has been described in Abyssinian cats. Tumorlike amyloid nodules and subcutaneous amyloid have been reported in horses. In general, however, amyloid deposits are found in the liver, spleen, and kidneys, particularly within glomeruli. In humans, amyloid fibrils are deposited in the neurons of patients with Alzheimer's disease. Misfolded prion proteins appear to be the cause of spongiform encephalopathies such as "mad-cow" disease. Prions, the infectious proteins responsible for spongiform encephalopathies, are protease-resistant forms of a cellular protein, PrP^c, that is important for normal macrophage functions. These prion proteins play a role in resistance to intracellular bacteria such as *Brucella*.

It is of interest to note that even reactive amyloidosis is somewhat "transmissible," since inoculation of AA proteins into an animal will hasten the development of amyloidosis. They seem to act by providing a substrate upon which other misfolded proteins can be deposited. Similarly, silk fibers formed from a pro-tein composed of β-sheets may also trigger amyloidosis when injected into mice.

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