Inflammation is a fundamental early defense mechanism against infection and the initiator of basic tissue repair. In sharp contrast, prolonged, uncontrolled inflammation may have detrimental effects, including excessive pain, immunosuppression, organ dysfunction, and death. This suggests that patient well-being is heavily dependent upon an appropriately balanced inflammatory response. Inflammation may affect every aspect of clinical decision making and surgical patient management. The timing of surgical intervention may be determined by the amount of local or systemic inflammation present. Intraoperative selection of various approaches, techniques, and even materials often hinges on attempts to minimize trauma and inflammation. In the immediate postoperative period, the inflammation experienced by a patient will dictate therapeutic decisions and even prognosis. Finally, long-term recovery will be affected by residual inflammation and any concurrent inflammatory conditions. Therefore it is imperative that surgeons have in-depth knowledge of the triggers and mediators, as well as the innate compensatory mechanisms and therapeutic targets that regulate the inflammatory response.

ACUTE INFLAMMATION

The classic macroscopic signs of acute inflammation include rubor (redness, erythema), calor (heat), dolor (pain), and tumor (swelling, edema), resulting in loss of function. These signs are the manifestations of increased localized blood flow and exudation of protein-rich fluid that occur within minutes of exposure to an inflammatory stimulus to promote the cleanup and repair of damaged tissue. This pathophysiologic response is regulated and counterregulated by a complex orchestra of mediators that will be the focus of this text.

The Acute Vascular Response

Vasodilation  Upon initial injury, local arterioles may undergo rapid, inconsistent, and transient vasoconstriction to provide some immediate hemostasis. Vasoconstriction is stimulated by vasoactive agents such as catecholamines, serotonin, bradykinin, and prostaglandins that are released from surrounding tissues and by norepinephrine released by the sympathetic nervous system. Within minutes, vasodilation and the opening of new capillary beds occur, leading to increased blood flow and local delivery of soluble mediators and inflammatory cells. Vasodilation is induced by vascular smooth muscle changes mediated by nitric oxide, histamine, leukotrienes, prostaglandins, and complement factors. Concurrently, lymphatic vessels proliferate to accommodate drainage of edema and serve as an avenue for microbes to reach local lymph nodes. Although these initial responses may be beneficial, an imbalance of mediators may promote widespread vasodilation, systemic hypotension, and even shock, as seen in severe sepsis.

Permeability  Vasodilation is followed by an increase in vascular permeability caused by various mechanisms. An increase in the number and size of intracellular endothelial gaps in the venules is mediated by histamine and serotonin. These transcytoplasmic channels (vesiculovacuolar organelles) allow transcytosis of plasma products to the site of inflammation. However, some molecules cannot traverse these channels and thus use other mechanisms. For example, interendothelial gaps, which are used by very large plasma molecules and mediators, may form, and endothelial cell retraction may occur; both processes are stimulated by hypoxia, endothelial injury, cytokines, or other inflammatory mediators. Vascular permeability is also increased by direct trauma and leukocyte-mediated endothelial damage.

Increased vascular permeability is accompanied by serum protein loss, resulting in decreased intravascular osmotic pressure, increased blood viscosity, and subsequent increases in interstitial osmotic pressure. These pathophysiologic changes, combined with early increases in hydrostatic pressure, lead to edema fluid accumulation in the interstitial space. Edema facilitates the delivery of beneficial soluble factors (antibodies and acute phase proteins) to the site of inflammation. However, along with alterations in tissue pH and oxygenation, edema induces localized pain and may precipitate loss of function.

Stasis  Fluid loss leads to hemoconcentration, described as an increased erythrocyte concentration in the local vasculature. Combined with decreased hydrostatic pressure, hemoconcentration leads to congestion and intravascular stasis, which allows increased contact time among erythrocytes, leukocytes, and the vascular endothelium, leading to the next stage of acute inflammation.
SECTION I • Surgical Biology

Leukocyte Extravasation  As hemostasis develops, leukocytes begin to marginate along the blood vessel walls to interact with endothelial cells, primarily in the systemic postcapillary venules and the pulmonary capillaries (Figure 1.1).252 This intimate association promotes weak, transient interactions between the glycoprotein cell adhesion molecules called selectins on endothelial cells and their corresponding ligands on inflammatory leukocytes, for example, the carbohydrate ligand Sialyl-Lewis X.4 The three most commonly described are designated E-, P-, and L-selectins according to their surface expression on endothelial cells, platelets, and leukocytes, respectively. In particular, E-selectins are upregulated by proinflammatory cytokines and bind receptor molecules on slow-moving, marginated leukocytes.113 Initially the weak endothelial-leukocyte interactions are susceptible to shear stress from blood flow. As the leukocytes roll along the endothelium at a velocity proportionate to blood flow, the affinity of their interactions with endothelium increases as the weak bonds break and re-form.252 Leukocyte adhesion to the vascular wall occurs through high affinity bonds formed by integrins on the leukocyte surface. Integrins, proteins composed of α (CD11a, CD11b, CD11c) and β (CD18) subunits,252 have expression and binding affinity that are increased by proinflammatory mediators.23 Corresponding adhesion molecules on endothelial cells are normally expressed at low levels but are upregulated after exposure to inflammatory mediators, including cytokines, coagulation factors, and histamine.22 As a specific example, the intercellular adhesion molecule (ICAM)-1 on endothelial cells interacts with the integrins, lymphocyte function-associated antigen-1 (LFA-1, also known as CD11a/CD18), and macrophage antigen-1 (Mac-1, also known as CD11b/CD18). Overall, adherence halts the progression of leukocytes within the circulation, making them available for recruitment to the site of inflammation. Genetic deficiencies in adhesion molecules inhibit local leukocyte recruitment during inflammation, leading to recurrent bacterial and fungal infections accompanied by peripheral leukocytosis.166

Once adhered, leukocytes migrate through a process termed diapedesis. Although transcellular migration is possible, diapedesis occurs through the interendothelial junctions of postcapillary venules, facilitated by endothelial cell retraction and cell adhesion molecules.223,241 In response to molecular stimuli, adhesion molecules (e.g., ICAM-2) are expressed at concentrated levels near interendothelial cell junctions, where they interact with leukocyte integrins and facilitate transmigration.231 In addition, platelet-endothelial cell adhesion molecule (PECAM)-1, which resides on endothelial cells, facilitates leukocyte transendothelial migration and transmigration through the basement membrane (see Figure 1.1). Once through the endothelial barrier, leukocytes must penetrate the basement membrane through a complex process involving both adhesive and proteolytic events.272

After reaching the interstitial space, leukocyte migration occurs along chemical gradients of exogenous (bacterial byproducts) and/or endogenous (complement components,
Macrophages are integral products) recruit neutrophils to the site of injury and may attractants (cytokines, complement components, bacterial oxygen species and other inflammatory mediators and to tors and cytokines is induced. Both processes allow primed independent mechanisms.

Neutrophils Extracellular traps. Neutrophil extracellular traps are cals and other toxic molecules, and the formation of neutrophil extracellular traps. Neutrophils provide local killing and degradation of bacterial macromolecules via phagocytosis, release of superoxide radicals and other toxic molecules, and the formation of neutrophil extracellular traps.

Within the neutrophil, there are multiple types of granules. The most prominent granule type used to identify neutrophils microscopically is the large azurophil (primary) granule, which contains microbicidal polypeptides such as myeloperoxidase, defensins, lysosome hydrolases, and neutral proteases. Smaller, specific (secondary) granules contain metalloproteases. Gelatinase (tertiary) granules and secretory vesicles contain preformed receptors for enhanced cellular communication.

In addition, neutrophils produce several proinflammatory cytokines (interleukin-1, IL-1, tumor necrosis factor-alpha, TNF-α) that stimulate further inflammation. Neutrophils may have complications, including secondary infections.

Cellular Components

The acute inflammatory response described earlier is often perceived as a product of neutrophil function. However, many immunocytes, including monocytes, lymphocytes, mast cells, natural killer cells, and dendritic cells, are activated during the inflammatory response. Cellular actions are choreographed by the activity of multiple mediators generated by these immunocytes and other cell types like vascular endothelium and parenchymal cells. In response, the cells are responsible for the production of additional mediators functioning to enhance, sustain, and/or resolve the inflammatory response. Although entire volumes have been written about each of these cellular components, this chapter aspires to give a brief description of only a few.

Neutrophils In the majority of mammalian species, neutrophils are the most numerous circulating leukocyte and a primary participant in inflammatory reactions. Typically neutrophils are the first migratory cells to arrive and take a predominant role in acute inflammation, with peak populations present during the first 24 to 48 hours. Numerous chemoattractants (cytokines, complement components, bacterial products) recruit neutrophils to the site of injury and may also activate the neutrophils to perform several functions. Neutrophils provide local killing and degradation of bacterial macromolecules via phagocytosis, release of superoxide radicals and other toxic molecules, and the formation of neutrophil extracellular traps. Neutrophil extracellular traps are composed of cell-free DNA and other antimicrobial peptides, including histones, which trap microorganisms, ensuring a local high concentration of granule enzymes for destruction.

Evidence also suggests that neutrophil extracellular traps promote clotting through platelet-dependent and platelet-independent mechanisms. Within the neutrophil, there are multiple types of granules. The most prominent granule type used to identify neutrophils microscopically is the large azurophil (primary) granule, which contains microbicidal polypeptides such as myeloperoxidase, defensins, lysosome hydrolases, and neutral proteases. Smaller, specific (secondary) granules contain metalloproteases. Gelatinase (tertiary) granules and secretory vesicles contain preformed receptors for enhanced cellular communication.

In addition, neutrophils produce several proinflammatory cytokines (interleukin-1, IL-1, tumor necrosis factor-alpha, TNF-α) that stimulate further inflammation. Neutrophils are a major, early source of proinflammatory cytokines. A conditional source of macrophages, circulating monocytes can extravasate in response to chemotaxins, including cytokines, fibronectin, elastin, complement factors (C3a, C5a), thrombin, and growth factors (e.g., platelet-derived growth factor [PDGF], transforming growth factor-beta [TGF-β]). Once in the tissue, monocytes can differentiate into macrophages and reside in the provisional fibrin-based extracellular matrix at a site of inflammation. Monocyte-derived macrophages can constitute the main macrophage type in inflammatory conditions. In addition to their production of macrophages, monocytes serve as short-lived effector cells that promote vascular regrowth in tissues.

Macrophages and Monocytes Macrophages are integral to the inflammatory response, wound debridement, and tissue repair. There are two types of macrophages: tissue-resident macrophages and monocyte-derived macrophages. Tissue macrophages are constitutively present, sentinel cells that arise early in embryogenesis, independent of monocytes. Tissue-resident macrophages are responsible for early recognition of inflammatory stimuli and are a major, early source of proinflammatory cytokines. A conditional source of macrophages, circulating monocytes can extravasate in response to chemotaxins, including cytokines, fibronectin, elastin, complement factors (C3a, C5a), thrombin, and growth factors (e.g., platelet-derived growth factor [PDGF], transforming growth factor-beta [TGF-β]). Once in the tissue, monocytes can differentiate into macrophages and reside in the provisional fibrin-based extracellular matrix at a site of inflammation.

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Macrophage polarization refers to the ability of macrophages to assume two distinct functional phenotypes: M1 and M2. M1 macrophages are classically activated by infectious agents or proinflammatory cytokines (interferon-gamma [IFN-γ] or TNF-α). They debride the affected site by phagocytosis of foreign material, pathogens, and damaged cells. Activated M1 macrophages produce proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and prostaglandins, enhancing the inflammatory response. Conversely, M2 macrophages are activated in response to antiinflammatory cytokines (IL-4, IL-13, and IL-10). They aid in wound repair and healing by secreting growth factors like PDGF or TGF-β, which stimulate fibroblasts to produce collagen, further dampening the inflammatory response. Both M1 and M2 macrophages secrete enzymes like collagenases and elastases to dissolve the extracellular matrix, facilitating phagocytosis and remodeling, respectively.

Although the classification is useful conceptually, the reader should be aware that the M1/M2 classification concept is an oversimplification of a continuum of macrophage phenotypes and expression profiles.

Similar to macrophages, monocytes also have heterogeneous subpopulations with discrete cytokine profiles that serve to modulate the inflammatory environment. If not appropriately stimulated for extravasation and differentiation, monocytes in circulation are relatively short-lived and undergo spontaneous apoptosis on a daily basis. By comparison, tissue macrophages are more persistent with life spans ranging from months to years. Alterations or dysregulations in subpopulations of either have been implicated in numerous disease conditions in people, including sepsis, chronic inflammatory lung disease, and atherosclerosis.

In addition to their role in debridement and tissue remodeling, macrophages also influence the adaptive immune system as antigen presenting cells that interact with lymphocytes.
**Lymphocytes** Although lymphocytes evoke attention to acquired immunity, it is now evident that they play a role in early, protective inflammatory responses. In particular, the helper (CD4+) T-cells and the cytotoxic (CD8+) T-cells are major components of cell-mediated immunity. CD4+ cells further differentiate into Th-helper-1 (Th-1) and Th-helper-2 (Th-2) cells. These T-cell characterizations are defined by their respective cytokine profiles. Under the influence of IFN-γ and IL-12, T-cells differentiate into Th-1 cells that produce a characteristic cytokine profile, including IFN-γ and IL-2. Th-1 cells exert major influences on macrophages by maximizing the bacterial killing potential of macrophages and stimulating proliferation of cytotoxic T-cells. In sepsis the early loss of T-cells due to apoptosis contributes to poor outcome, which can be reversed by T-cell replacement. The IFN-γ produced by Th-1 cells also stimulates immunoglobulin G (IgG)2a production by B-cells. Although the mechanisms are not completely understood, severe tissue trauma can result in a bias toward Th-2 responses. Increased production of IL-4 and IL-10 actually inhibits Th-1 cell proliferation and may predispense toward infectious complications. Both play a pivotal role in immunomodulation and functionally antagonize each other. However, their molecular mechanisms are less understood than the traditional T-cell classes. Formerly known as the suppressor T-cells, Tregs function to maintain tolerance to self-antigens and downregulate the actions of other T-cells to reduce inflammation. Increased Treg expression or numbers have been implicated in tumor progression, metastasis, and an overall poor prognosis. In contrast, Th-17 cells are pro-inflammatory, producing IL-17, IL-17F, and IL-22. Th-17 cells serve to combat extracellular fungal, bacterial, and mycobacterial pathogens by secreting chemokines and colony-stimulating factors to facilitate cellular recruitment, filling a critical void in host immunity not covered by the Th-1/Th-2 paradigm. If not appropriately checked, however, Th-17 cells are major contributors to inflammatory autoimmune disease. It is evident that all types of T-cells and their products must be balanced for an appropriate inflammatory response.

**Mast Cells** Mast cells are ubiquitously distributed in all organs and degranulate in response to physical trauma, complement factors, microbial products, or neuropeptides. They are the primary source of histamine during acute inflammation. In addition, they release other proinflammatory mediators like serotonin, leukotrienes, prostaglandin metabolites, heparin, and cytokines. There is growing evidence that these cells actively participate in neurogenic inflammation, but the mechanisms of this are still unclear. Overall, mast cell degranulation enhances the local inflammatory response.

**Endothelial Cells** Endothelial cells are a dynamic cell population that composes the inner lining of the vasculature and lymphatic systems. During an inflammatory response, they can increase vascular permeability, promote extravasation of circulating immunocytes, and alter coagulation. Cytokines (e.g., IL-1β and TNF-β) and bacterial products such as endotoxin activate endothelial cells via a two-stage process, similar to the one previously described for neutrophil activation. Endothelial cells first undergo a rapid, transient upregulation of preformed and stored von Willebrand factor (vWF) and P-selectin, followed by a de novo gene expression of proinflammatory cytokines (TNF-α, IL-8, IL-1), chemotactants (monocyte chemoattractant protein [MCP]-1), and adhesion molecules (E-selectin, P-selectin, ICAM-1, vascular cell adhesion molecule [VCAM]-1). These alterations ensure the extravasation, migration, and subsequent accumulation of leukocytes and lymphocytes at sites of injury. In certain circumstances, endothelial cells can also express major histocompatibility complex class II to act as antigen presenting cells, effectively bridging innate and adaptive inflammatory responses.

**INFLAMMATORY STIMULI**

Inflammation is caused by an array of offending agents, including trauma, infection, foreign material, caustic chemicals, allergens, and autoimmune disease. These agents trigger relatively generic responses, suggesting that molecular triggers are fairly redundant, or that diverse signals share common molecular pathways to produce a similar end result. To maintain focus on surgery and inflammation, this text will primarily discuss traumatic and infectious causes of inflammation.

**Alarm Signals: Pathogen-Associated Molecular Patterns and Danger-Associated Molecular Patterns**

Multicellular organisms use an evolutionarily conserved system to alert the body to infection or cellular damage. The warning molecules, either exogenous or endogenous, incite intracellular signaling cascades that eventually affect basic cell functions (Figure 1.2). Pathogen-associated molecular patterns are highly conserved microbial molecules, recognized as foreign to the host. Such patterns include lipopolysaccharide, lipoteichoic acid, peptidoglycan, and microbial oligonucleotides. In contrast, danger-associated molecular patterns are endogenous molecules such as fibrinogen, which alert the body to cellular damage initiated by infectious or noninfectious agents. An example of a danger-associated molecular pattern is the intracellular molecule high-mobility group B1, which is released with cellular damage or necrosis and is an important mediator of late-stage sepsis. Heat shock proteins are intracellular chaperones that normally regulate proper protein folding. First identified from cells subjected to thermal stress, heat shock proteins are known to be produced in response to other stimuli and are found in the circulation after trauma and surgery. Heat shock protein 60 and heat shock protein 70, produced by activated monocytes, stimulate other cells in the innate immune system. Collectively, the pathogen-associated molecular patterns and danger-associated molecular patterns signal the immune system by interacting with cell surface receptors.

**Pattern Recognition Receptors**

Pattern recognition receptors are expressed on the cell surface or within the intracellular compartment; however, soluble forms may be found in bodily fluids. The group includes toll-like receptors, scavenger receptors, mannose receptors,
MEDIATORS OF INFLAMMATION

Vasoactive Amines

Vasoactive amines, namely histamine and serotonin, are the primary mediators of the acute inflammatory response. Their downstream effects contribute to short-lived homeostatic alterations, including vasodilation, increased vascular permeability, and nonvascular smooth muscle contraction. These alterations allow cellular mediators of inflammation to gain access to sites of injury and contribute to wound healing. Inappropriate release of vasoactive amines can result in a spectrum of hypersensitivity reactions from cutaneous allergic reactions to anaphylactic shock.

Histamine

Histamine is a major vasoactive amine that is stored preformed within cellular granules and therefore is among the first active mediators released during acute inflammation. It is primarily produced and released by mast cells located in the connective tissue immediately adjacent to blood vessels, although histamine is also produced by basophils, platelets, and other cell types located in the same tissue or within the bloodstream. Histamine is released in response to a variety of stimuli, including physical injury, antibody binding (primarily allergic reactions), and complement protein binding (C3a and C5a). Additionally, neuropeptides and some cytokines may also trigger histamine release. Histamine

C-type lectin-like domain–containing receptors, peptidoglycan recognition receptors, and nucleotide-binding site–leucine-rich repeat receptors (Table 1.1). Many of these are promiscuous, binding to more than one alarm signal molecule. A single ligand may also bind more than one receptor, ensuring a robust and diverse response. The individual effects of pattern recognition receptor activation are too numerous to list; however, collectively they initiate the complex cellular responses that result in inflammation.

Toll-like Receptors

Toll-like receptors, arguably the most important and certainly the most studied pattern recognition receptor, are type 1 transmembrane proteins that initiate intracellular signaling cascades, which, in general, activate nuclear factor kappa B (NFκB) and result in altered gene transcription. Although many toll-like receptors have been discovered, nine are well characterized as of this writing (see Table 1.1). Toll-like receptors play a central role in the release of inflammatory cytokines from the innate immune response to microbial structures such as peptidoglycan and lipoteichoic acid. Toll-like receptor 4 (TLR4), in particular, is a major receptor for lipopolysaccharide (endotoxin). In concert with the receptor CD14 and the soluble mediator lipopolysaccharide-binding protein, TLR4 activation increases expression of numerous proinflammatory mediators and modulates the further expression of other toll-like receptors.
Although a gross oversimplification, cells exposed to pathogen-associated molecular patterns and danger-associated molecular patterns produce a cascade of cytokines, beginning with TNF-α and IL-1β, followed by IL-6 and the chemotactic cytokines. This leads to production of acute phase proteins, leukocyte recruitment, and release of other mediators that modulate inflammatory responses (see Figure 1.2).

**Proinflammatory Cytokines** Although a gross oversimplification, cells exposed to pathogen-associated molecular patterns and danger-associated molecular patterns produce a cascade of cytokines, beginning with TNF-α and IL-1β, followed by IL-6 and the chemotactic cytokines. This leads to production of acute phase proteins, leukocyte recruitment, and release of other mediators that modulate inflammatory responses (see Figure 1.2).

**Tumor Necrosis Factor** Tumor necrosis factor (TNF-α) is one of the most studied cytokines. Originally called cachectin, TNF-α was first described for its remarkable antitumor activity and association with cachexia in chronic disease states. Activated M1 macrophages are a major source of TNF-α; however, other activated cell types will also upregulate TNF-α production during inflammatory responses. TNF-α is a membrane-bound surface protein, cleaved by metalloproteases, that is released in soluble form. It interacts with two known receptors, TNFR1 and TNFR2, which are found on numerous cell types, suggesting that TNF-α mediates an array of effects.

In response to a stimulus, TNF-α concentration peaks quickly. Consequently, it may go undetected in some studies of inflammation, even after major surgical interventions. After its release, TNF-α initiates production of proinflammatory cytokines (e.g., IL-6), reactive oxygen intermediates, chemotaxis, and endothelial adhesion molecules; all facilitate the recruitment of cells at the site of inflammation. TNF-α causes a wide range of additional effects, including activation of natural killer cells, proliferation of cytotoxic T-cells, and T-cell apoptosis. These effects may be inherently considered when physiologic responses are compared across species.

**Cytokines**

The term cytokine refers to a very diverse group of small, soluble proteins that act as intercellular messengers during a number of physiologic processes. The group includes tumor necrosis factors, interleukins, transforming growth factors, interferons, colony-stimulating factors, and others (see Figure 1.2). Once referred to as lymphokines, cytokines are actually produced by more than one cell type, and a single cell may produce several different cytokines. Secreted in small concentrations that quickly dissipate, cytokines generally exert their influence locally with autocrine or paracrine cellular effects but may disseminate and influence cells at distant sites. Cytokines interact with cell surface receptors to initiate intracellular signaling pathways that influence cell functions and the production of more cytokines. Several cytokines may act on the same receptor, and a given cytokine may initiate a response at multiple receptors. This promiscuity in receptor affinity ensures the maintenance of innate immune responses.

Cytokines are difficult to categorize because of their diversity. Classifications based on cell of origin, structural homology, molecular mechanisms, receptors, and end functions have been described. None of these systems provide well-demarcated groupings because redundancy and pleiotropism are inherent characteristics of cytokines. Here the cytokines will be grouped by a functional classification. Proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) increase the innate immune response, and antiinflammatory cytokines (e.g., IL-10, IL-1 receptor antagonist [IL-1ra]) attenuate the responses. However, it is important to note that cytokine function may depend upon timing, concentration, and association with other cytokines, highlighting the complexity of immune responses. This discussion will include those cytokines classically regarded as integral to the acute inflammatory response. For comprehensive listings, readers are referred elsewhere.

### Proinflammatory Cytokines

Cytokines are difficult to categorize because of their diversity. Classifications based on cell of origin, structural homology, molecular mechanisms, receptors, and end functions have been described. None of these systems provide well-demarcated groupings because redundancy and pleiotropism are inherent characteristics of cytokines. Here the cytokines will be grouped by a functional classification. Proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) increase the innate immune response, and antiinflammatory cytokines (e.g., IL-10, IL-1 receptor antagonist [IL-1ra]) attenuate the responses. However, it is important to note that cytokine function may depend upon timing, concentration, and association with other cytokines, highlighting the complexity of immune responses. This discussion will include those cytokines classically regarded as integral to the acute inflammatory response. For comprehensive listings, readers are referred elsewhere.

**Serotonin** Serotonin (5-hydroxytryptamine) has actions similar to histamine; however, it is not a major mediator in the acute inflammation response of human beings or other nonrodent species. In mice and rats, serotonin is released from mast cells, basophils, and some neuroendocrine cells during platelet aggregation. Therefore the effects of serotonin on acute inflammation are species dependent and must be considered when physiologic responses are compared across species.
counteracted in vivo by the release of tumor necrosis factor receptors from the cell surface. These tumor necrosis factor soluble receptors are found constitutively at low levels in the blood but are increased in inflammatory conditions such as sepsis. The solubilized receptors bind to TNF-α and effectively reduce the cytokine’s activity.

TNF-α has both beneficial and deleterious effects. When administered experimentally, it results in classic signs of endotoxic shock, including hypotension, metabolic acidosis, and death. Inhibition of TNF-α is protective in endotoxic shock. Conversely, TNF-α is necessary for protection from mycobacterial infection and blocking its activity increases mortality in septic human patients. As a major initiator of inflammation, TNF-α has been directly linked to a number of diseases, leading to interest in TNF-α as a therapeutic target. Corticosteroids are known to inhibit production of TNF-α; however, a more focused approach has been applied to specific diseases. Commercically available anti-tumor necrosis factor monoclonal antibodies and recombinant tumor necrosis factor soluble receptors have proven efficacious in human patients with Crohn’s disease and rheumatoid arthritis. Although the therapeutic inhibition of TNF-α has been associated with recrudescence of pulmonary mycobacterial infections and infectious complications after orthopedic surgeries, the success of anticytokine therapy in the treatment of autoimmune diseases in human beings is believed to outweigh the risks.

**Interleukin-1** The term interleukin-1 (IL-1) denotes several cytokines produced by macrophages and other cell types. IL-1β is secreted as an inactive proform, which is cleaved by IL-1-converting enzyme, also known as caspase-1. However, genetically engineered mice deficient in IL-1-converting enzyme remain responsive to endotoxin, suggesting that redundancy exists between IL-1β and other cytokines. IL-1β complexes with a functional receptor called IL-1Rα and a third component, the IL-1 receptor accessory protein (IL-1RαcP), to initiate cellular signaling pathways. Another member of the IL-1 family of cytokines, IL-1ra, serves a counterregulatory function and is actually an antiinflammatory cytokine that competes with IL-1 for receptor sites. Genetically manipulated mice deficient in IL-1ra show exaggerated inflammatory responses, illustrating its importance in IL-1β regulation. Overall, the IL-1 family of cytokines demonstrates the intricacies of cytokine regulation, which may involve several layers of control, including production, processing, receptor availability, and accessory proteins.

The proinflammatory functions of IL-1β are similar to those of TNF-α, and these cytokines often work synergistically to further enhance inflammation and promote sepsis. In response to inflammatory stimuli, IL-1β mediates increases in production of proinflammatory cytokines, prostaglandins, and nitric oxide. These changes are manifested in host responses, including hypotension, fever, decreased white blood cell counts, hemorrhage, and pulmonary edema. Competitive inhibition of the IL-1 receptor improves survival after experimental administration of endotoxin. As with tumor necrosis factor, IL-1β has been implicated in a number of inflammatory diseases, including sepsis, Crohn’s disease, and rheumatoid arthritis.

**Interleukin-6** Interleukin-6 (IL-6) levels increase in virtually all inflammatory conditions. Produced by macrophages, T-cells, epithelial cells, and enterocytes, it plays a pivotal role in initiating hepatic synthesis of the acute phase protein and influences the proliferation of lymphocytes. In addition, IL-6 has a contradictory role, initiating compensatory antiinflammatory responses and downregulating proinflammatory cytokine production. In inflammatory states, plasma IL-6 level increases proportionately with the duration and severity of the condition. After surgical trauma, plasma levels are higher with invasive procedures as compared with laparoscopy. Plasma IL-6 levels have been used to predict postoperative infection, sepsis-associated mortality, and the recurrence of abdominal adhesions. Consequently, IL-6 is considered to be not only a mediator but also a diagnostic and prognostic biomarker of inflammation.

**Chemokines** During acute inflammation, chemokines peak shortly after TNF-α, IL-1β, and IL-6. Chemokines are the chemotactic cytokines responsible for attraction of cells across a concentration gradient during embryonic development, wound healing, angiogenesis, and inflammatory responses. More than 40 chemokines are known to be secreted by macrophages, endothelium, and other cell types. As with all cytokines, redundancy in cell specificity, receptor affinity, and function is noted among the chemokines. Therefore they are categorized into families according to structural placement of conserved cysteine residues (e.g., CXC chemokines have one amino acid separating two cysteine residues). Of the four chemokine families, CXC and CC chemokines contain members most actively involved in the proinflammatory response to trauma or infection. Within the CXC family a subgroup carries an ELR moiety (glutamine-leucine-arginine), conferring the ability to attract neutrophils, whereas an ELR-negative subgroup attracts mononuclear cells. Interleukin-8 (IL-8) is the archetypical neutrophil chemoattractant in the majority of mammals and, under the most recent nomenclature, is referred to as CXCL8. It is noteworthy that rodents commonly used in inflammation research do not express IL-8/CXCL8 but have several functional counterparts. IL-8/CXCL8 attracts neutrophils and has several other functions, including the upregulation of surface adhesion molecules, triggering degranulation of proteases, and increasing the production of other inflammatory mediators. As an inflammatory response progresses, additional chemokines, such as MCP-1/CCL2 and macrophage inflammatory protein (MIP-1α/CCL3), participate in the recruitment of monocytes, promoting a transition from active to chronic phases of inflammation. Over time, cellular recruitment slows as chemokines are degraded by enzymes and further production slows.

**Antinflammatory Cytokines** Theories once suggested that accumulation of proinflammatory mediators triggers production of counterregulatory factors that eventually created an antiinflammatory state. However, this is probably an oversimplification. Some antiinflammatory mediators are triggered by the initial inflammatory insult, whereas others are constitutively produced and help maintain homeostasis. Therefore the antiinflammatory cytokines are quite diverse. Some of these, including the aforementioned tumor necrosis factor soluble receptors and IL-1ra, inhibit the normal activity of proinflammatory cytokines. Others work by suppressing the production of proinflammatory mediators, either directly or indirectly. The desired outcome is a balance of proinflammatory and antiinflammatory mediators that protect the host from microbial invasion while promoting healing.

**Interleukin-10** Interleukin-10 (IL-10) is the archetypal antiinflammatory cytokine, produced primarily by CD4+ Th-2 T-cells, monocytes, and B-cells. It depresses the production of several proinflammatory cytokines and chemokines, including TNF-α, IL-1, IL-6, and IL-8, by inhibiting translocation of NFκB and promoting degradation of messenger RNAs. IL-10 downregulates the production of Th-1 cytokines, which are protective during microbial infection, and plays a role in limiting inflammatory responses to normal gut-associated bacteria. In addition, IL-10 promotes shedding of tumor necrosis factor receptors into the systemic circulation.
also inhibits antigen presentation by macrophages and dendritic cells. In a balanced immune response, IL-10 levels would be low during acute phase inflammation and would increase over time. IL-10 deficiencies have been reported in chronic inflammation, autoimmune diseases, and after transplantation surgeries, which may contribute to poor outcomes. Conversely, exogenous IL-10 has been used to reduce intestinal inflammation in human patients with Crohn’s disease. However, excess IL-10 can increase susceptibility to microbial infection and may influence survival. This illustrates that a fine balance of cytokines is necessary to ensure appropriate inflammatory responses.

**Lipid/Cell Membrane–Derived Mediators**

**Eicosanoids** Eicosanoids are lipid mediators rapidly synthesized de novo from cell membrane phospholipids. Arachidonic acid, their fatty acid precursor, is stored in the cell membranes of endothelial cells, leukocytes, and other cells; released by activated phospholipase A₂; and rapidly metabolized by the cyclooxygenase or lipoxygenase pathway (Figure 1.3). Arachidonic acid production is inhibited by glucocorticoids as a result of decreased phospholipase A₂ expression and upregulation of genes encoding antiinflammatory proteins.

**Prostaglandins** Prostaglandins are produced in the cyclooxygenase pathway, where arachidonic acid metabolism is catalyzed by the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is a constitutively expressed enzyme involved in homeostasis and is present in the majority of mature cells; COX-2 expression is induced by trauma, growth factors, proinflammatory cytokines, and other mediators. Prostaglandins mediate many inflammatory responses primarily through G protein–coupled receptors on a number of cell types. They are chemotactic agents that cause leukocyte recruitment and vasodilation, contributing to the pathogenesis of pain and fever during inflammation. Aspirin and nonsteroidal antiinflammatory drugs (e.g., carprofen, indomethacin) inhibit the cyclooxygenase enzymes. Selective inhibition of the inducible COX-2 while sparing the constitutively produced COX-1 has received a great deal of attention. It was initially believed that COX-1 inhibition caused gastric ulceration and thus should be spared. However, COX-2 inhibition alone increased the risk for cardiovascular and cerebrovascular events as seen in human clinical trials, probably through the as yet ill-defined role of COX-2 in vascular homeostasis. In addition, COX-2 may actually help resolve acute inflammation and heal gastric ulcers. Thus the use of selective COX-2 inhibitors for treating chronic inflammation has gone out of favor in human medicine. However, no compelling evidence suggests that dogs develop cardiovascular events with COX-2 inhibitor use. This, combined with the decreased incidence of gastric ulceration, makes COX-2 selective agents a good option in dogs.

**Figure 1.3** The arachidonic acid pathway. Arachidonic acid is metabolized by the cyclooxygenase pathway to produce prostaglandins or the lipoxygenase pathway to produce leukotrienes and proresolusion lipoxins. The inhibitory effects of several drugs on specific enzymes are denoted by a red X. COX, Cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid. (From Kumar V, Abbas A, Fausto N, et al.: Robbins and Cotran pathologic basis of disease, ed 8, Philadelphia, 2009, Saunders/Elsevier.)
neutrophil chemotaxis by blocking β1 integrin-mediated cell adhesion and decreasing P-selectin expression on endothelial cells.219 However, lipoxins A4 and B4 increase monocyte chemotaxis and activate monocyte-endothelial transmigration.169 The differential actions of lipoxins assist in the transformation from acute inflammation to a chronic, wound-repair state. Resolvins and protectins are also lipid-derived proresolution mediators; however, they are generated from two of the omega-3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid, respectively. They decrease inflammation by binding G protein–coupled receptors on leukocytes.248,249 This explains the antiinflammatory effects of fish oils that contain eicosapentaenoic acid and docosahexaenoic acid. Eicosapentaenoic acid also acts as a competitive inhibitor of arachidonic acid, which leads to decreased synthesis of the proinflammatory eicosanoids.135

Platelet-Activating Factor Similar to eicosanoids, platelet-activating factor is metabolized from cell membrane phospholipids by phospholipase A2. It is produced by numerous cells, including endothelial cells, neutrophils, platelets, macrophages, and eosinophils.21 The synthesis of platelet-activating factor is primarily modulated by mitogen-activated protein kinase (MAPK) intercellular signaling pathways, resulting in both free and cell membrane–bound forms. This expression is enhanced by multiple inflammatory mediators, including reactive oxygen species, histamine, and cytokines. Platelet-activating factor binds to G protein–coupled receptors found on various cell types.51,257 Where it stimulates arachidonic acid release and subsequently increases eicosanoid production. It exerts proinflammatory effects by increasing the affinity and avidity of the surface integrins on neutrophils to enhance their adhesiveness, motility, and degranulation.55,226 It causes platelet aggregation and degranulation,55 as well as eosinophil degranulation and production of reactive oxygen species.260 Other actions of platelet-activating factor include increased vascular permeability, bronchoconstriction, and pulmonary vasoconstriction.54,117 In vivo it is rapidly degraded by platelet-activating factor-acetylhydrolase, making this enzyme a potential therapy for inflammatory diseases.46,228

Reactive Oxygen Species Reactive oxygen species are unstable molecules that initiate chain reactions to perpetuate further reactive oxygen species production. Free radicals are reactive oxygen species with unpaired electrons that accept electrons from other molecules (proteins, lipids, carbohydrates) and consequently destabilize those molecules. This group includes the highly reactive hydroxyl radical and superoxide anion (O2·−).235 Non-free radicals are reactive oxygen species, such as hydrogen peroxide (H2O2), that have paired electrons but are nonetheless easily destabilized, resulting in further free radical production. Reactive oxygen species have diverse effects, including antibacterial defense, wound debridement, intracellular signaling, and pathologic tissue damage.229

In certain situations, reactive oxygen species are beneficial. Under homeostatic conditions, reactive oxygen species form during electron transport as part of mitochondrial respiration to generate adenosine triphosphate (ATP) for cellular processes. Phagocytic cells also produce reactive oxygen species within phagocytic vesicles in a process known as the respiratory or oxidative burst.115 Within the lysosome or phagolysosome, oxygen consumption increases when the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalyzes the transfer of electrons from NADPH to oxygen, generating superoxide. Superoxide may be metabolized to hydrogen peroxide through the action of superoxide dismutase (SOD). Another reactive oxygen species–generating enzyme found in neutrophil granules,
Nitric oxide is synthesized from the amino acid L-arginine by the enzyme nitric oxide synthase. Three forms of nitric oxide synthase are known. Endothelial-derived nitric oxide synthase and neuronal-derived nitric oxide synthase are constitutively produced, and inducible nitric oxide synthase is produced in response to cytokines and other inflammatory mediators (Figure 1.4). Inducible nitric oxide synthase can be expressed by a variety of cell types, including hepatocytes, keratinocytes, macrophages, respiratory epithelium, and neutrophils, and inflammatory conditions such as sepsis lead to upregulation of inducible nitric oxide synthase. Regulated vascular tone is a primary physiologic function of nitric oxide because it produces vasodilatation by diffusing into smooth muscle cells and indirectly initiating intracellular signaling events, leading to smooth muscle relaxation. In addition, nitric oxide antagonizes the vasoconstrictive effects of angiotensin II, endothelins, and reactive oxygen species; therefore it is a major contributor to early-stage vasodilation.

Nitric oxide exhibits both antiinflammatory and proinflammatory effects, depending on concentration and mode of production. In general, constitutive nitric oxide induces protective effects, which maintain homeostasis in the vascular and immune defense. Nitric oxide decreases platelet aggregation and leukocyte adhesion. Basal levels of nitric oxide also have protective antiinflammatory effects on the gastrointestinal tract. In addition, constitutive nitric oxide suppresses the action of NFκB, a transcription factor necessary for the expression of inducible nitric oxide synthase. In an inflammatory state, proinflammatory cytokines and bacterial metabolites decrease the production of constitutive nitric oxide synthase, which allows activation of NFκB and leads to an increase in inducible nitric oxide synthase in monocytes/
Hydrogen Sulfide

Hydrogen sulfide gas is produced in tissues during cysteine metabolism, mediated by two enzymes, cystathionine β-synthase and cystathionine γ-lyase. It is also a metabolic product of bacterial flora in the intestine. Acting at ATP-dependent K+ channels, hydrogen sulfide relaxes smooth muscles and induces vasodilation. Initial investigations using animal models of inflammation and sepsis suggested that hydrogen sulfide promotes inflammation, whereas inhibitors of hydrogen sulfide synthesis decrease levels of proinflammatory cytokines. More recent work strongly suggests that hydrogen sulfide has primarily antiinflammatory properties and promotes resolution of inflammation. This is largely attributed to the suppression of interactions between leukocytes and endothelial cells, resulting in decreased leukocyte recruitment. Blockade of hydrogen sulfide–producing enzymes enhances leukocyte recruitment. Of note, nonsteroidal antiinflammatory drugs suppress cystathionine γ-lyase, which in turn reduces hydrogen sulfide levels and contributes significantly to nonsteroidal antiinflammatory drug–associated gastritis. Additional studies are needed to fully evaluate the therapeutic uses of hydrogen sulfide–releasing compounds.

Acute Phase Proteins

The acute phase reaction is an initial response to inflammatory stimuli and functions as a mechanism to restore normal homeostasis. Its hallmarks are fever, leukocytosis, and changes in serum concentrations of acute phase proteins. Acute phase proteins are those proteins whose concentrations change significantly in response to inflammation. IL-6 works synergistically with other cytokines (IL-1, interferon-gamma [IFN-γ], and TNF-α) to stimulate the transcription and release of these proteins from hepatocytes. IL-1, meanwhile, can inhibit the production of some acute phase proteins. Other such proteins can be produced extrahepatically by neurons, monocytes, and lymphocytes. It is important to remember that the level of expression and the relative importance of specific acute phase proteins can differ widely from species to species.

Negative Acute Phase Proteins

Negative acute phase proteins are proteins active in regulating homeostasis that decrease in concentration by at least 25% during an inflammatory response. Theoretically, decreased production of proteins irrelevant to immediate host defense leads to increased availability of biologically active molecules necessary for production of more pertinent proteins. Albumin is the primary negative acute phase protein in most species. Other negative acute phase proteins include transferrin, apolipoprotein A, retinol-binding protein, cortisol-binding protein, and transthyretin.

Positive Acute Phase Proteins

Positive acute phase proteins show an increase in plasma concentration by at least 25% during an inflammatory response. In response to proinflammatory cytokines, their levels increase within a few hours, peak within 24 to 48 hours, and remain elevated as long as inflammatory stimuli persist. In general, the role of acute phase proteins is to enhance protective host functions by minimizing tissue damage and enhancing repair processes after infection, trauma, or stress. In addition to these functions, acute phase proteins may be used as diagnostic or prognostic markers for early identification of inflammation and prediction of outcomes.

C-Reactive Protein

C-reactive protein is a positive acute phase protein and a member of the pentraxin family. It binds...
to phosphocholine, ribonucleoproteins, and phospholipids, thus binding foreign bacteria as well as necrotic or apoptotic cells. Bacteria-bound C-reactive protein promotes activation of complement, contributing to host defense. It regulates leukocyte infiltration by inhibiting neutrophil chemotaxis and downregulating L-selectin on the neutrophil surface. C-reactive protein inhibits the respiratory burst in neutrophils, but activates macrophages to produce IL-1β, IL-6, TNF-α, and tissue factors, which activate the extrinsic clotting cascade. This may actually provoke disseminated intravascular coagulation and thrombosis during an inflammatory event. Obviously, C-reactive protein pathophysiology is complex and inconsistent, because it induces both proinflammatory and antiinflammatory responses.

C-reactive protein is an important analyte that provides diagnostic information on inflammatory status in human beings. Although less characterized in veterinary species, commercial assays for canine C-reactive protein may be useful. In particular, C-reactive protein levels, along with the percentage of band neutrophils, have been used to predict the presence of pyometra in dogs. However, unlike in human beings, horses, and dogs, C-reactive protein does not appear to be highly responsive in cats, limiting its diagnostic value in that species.

**Serum Amyloid A** Serum amyloid A is produced in macrophages and endothelial cells, as well as in hepatocytes. Although its concentrations may increase 1000-fold during inflammatory conditions, the magnitude of the response is disease and species dependent. A majority of its isoforms found in blood become components of high-density lipoprotein. Thus it is speculated that serum amyloid A aids in cholesterol clearance from macrophages after phagocytosis of damaged cell membranes. Free serum amyloid A stimulates the production of proinflammatory cytokines (TNF-α, IL-1β, IL-8) from leukocytes, particularly neutrophils. In addition, it increases IL-12, important for Th-1 antimicrobial responses, and IL-23, which plays a role in chronic and autoimmune inflammation in monocytes. Serum amyloid A is a chemoattractant for T-cells, monocytes, and neutrophils and upregulates adhesion molecule expression. Conversely, it mediates some antiinflammatory effects by decreasing PGE2 production, platelet activation, and oxidative bursts of neutrophils. Serum amyloid A has been investigated as an inflammatory marker in cats, in which its levels increased rapidly at approximately 3 to 6 hours after ovariohysterectomy and increases to significantly high levels in conditions like injury, renal failure, and infectious diseases.

**Serum Amyloid P** Serum amyloid P is structurally similar to C-reactive protein and, like C-reactive protein, is also in the pentraxin family. It is the major acute phase protein in rodents. Also similar to C-reactive protein, it binds bacteria, apoptotic cells, and DNA, which increases complement deposition and phagocytosis. Serum amyloid P binds endotoxin and has been proposed as a treatment for sepsis. In addition, it decreases fibrocyte differentiation and therefore may have a protective role against fibrotic disease. Evidence indicates that serum amyloid P inhibits autoantibody production, preventing antinuclear autoimmunity.

**Complement Proteins** The complement system comprises more than 30 serum proteins and cell surface receptors that play an integral role in opsonization, phagocytosis, chemotaxis, and active cell lysis (Figure 1.5). Complement proteins are hepatically synthesized proteases that circulate in their inactive form. Upon activation, complement components are cleaved into the active protease designated “b,” and subsequent breakdown products facilitate several aspects of inflammatory responses as well as pathogen removal via phagocytosis and membrane attack complex (MAC) production.

and a smaller, soluble mediator, designated “a.” Complement activation occurs via three different pathways: classical, lectin, and alternative. Immune complexes activate the classical pathway. Lectin pathway activation occurs via interaction of hepatically synthesized lectin proteins (e.g., mannose-binding lectin) with surface carbohydrates on pathogens. The alternative pathway is initiated by contact with foreign microbes. Although they have distinct activation mechanisms, all complement pathways merge at the cleavage of C3 to form C3b. When C3b forms, it binds to the appropriate enzyme, either classical C3 convertase (C4b2a) or alternative C3 convertase (C3bBb). These molecular complexes cleave C5 into C5a and C5b. C5b then binds to C6, C7, C8, and C9 in the terminal complement pathway to form the membrane attack complex. The membrane attack complex inserts into the lipid bilayer of target cell membranes and creates channels, leading to dysregulation of cellular homeostasis and eventual lysis. This process is beneficial because it destroys intracellular pathogens but may also lead to unwanted tissue destruction.

Other cleavage products of the complement cascade play important roles in modulating the immune response. The C3a, C4a, and C5a components are anaphylatoxins that enhance leukocyte chemotaxis, inflammation, and generation of oxygen free radicals. C3a is a potent activator of eosinophils and mast cells, which leads to the release of proinflammatory and vasoactive mediators. It modulates the humoral immune response by suppressing release of IL-6 and TNF-α from activated B-cells and subsequently decreasing the polyclonal antibody response. As such an integral mediator, C3a has been suggested as a potential biomarker and prognostic indicator in human trauma cases. C5a is a more potent inducer of smooth muscle responses compared with C3a and C4a. It is also a strong chemotactant for monocytes and neutrophils. Phagocytosis by these cells is enhanced by complement factors acting as opsonins. The C opsonins coat target cells, allowing direct interaction and ingestion by phagocytes bearing the appropriate complement receptors. In addition, C5a activates the generation of reactive oxygen species, which aid in bacterial killing. In fact, absence of C5a increases susceptibility to certain pathogens.

The complement system is tightly regulated at multiple levels by both plasma- and membrane-bound proteins. Overproduction of complement factors during conditions such as sepsis can induce a wide variety of tissue damage, leading to multiple organ failure. Proper regulation is integral to preventing the destructive effects in surrounding cells and tissues.

There is evidence of significant overlap between the complement system and the coagulation cascade. As early as the late 1970s–1980s, in vitro studies supported complement factor enhancement of thrombin-mediated platelet aggregation. Other studies demonstrate that coagulation proteases can initiate complement anaphylatoxin and upstream mediator formation. Although more research is needed to completely delineate these pathways, it is clear that these two systems have complex interactions and redundant mechanisms to maintain homeostasis and control inflammation.

**Coagulation Factors** The coagulation cascade aims to balance the vascular response during times of perturbation. Extremes such as hemorrhage or excessive clotting can lead to deleterious outcomes for a patient, including disseminated intravascular coagulation, hemorrhagic shock, and thromboembolic disease. Appropriate coagulation requires coordination of a complex cascade of serine proteases involving two pathways: the intrinsic pathway and the extrinsic pathway. The intrinsic pathway is activated when factor XII (Hageman factor) contacts a negatively charged surface, and the extrinsic pathway is initiated by activation of factor VII by tissue factor. Both pathways culminate at the conversion of prothrombin to thrombin, which then mediates the conversion of fibrinogen into fibrin and subsequent clot formation. Normal hemostasis results when procoagulant and anticoagulant factors are in equilibrium, much like the necessary balance between proinflammatory and antiinflammatory factors.

The fact that inflammation and coagulation are intertwined complicates the balancing act needed to achieve homeostasis. The coagulation system most notably exerts proinflammatory effects through four protease-activated receptors. On endothelium, activation of protease-activated receptors by thrombin promotes synthesis of prostaglandins, nitric oxide, and platelet-derived growth factor (PDGF). Thrombin also stimulates release of proinflammatory cytokines, recruitment of natural killer cells, and macrophage production of chemokines. Reciprocally, inflammation affects hemostasis by enhancing procoagulant activity, which may increase thromboembolic events. For example, inflammatory cytokines upregulate expression of von Willebrand factor (vWF) on vascular endothelium. In addition, conditions such as sepsis increase endothelial tissue factor, activating the extrinsic clotting cascade. Increased levels of plasma tissue factor correlate with the development of disseminated intravascular coagulation. Also in inflammatory disease, circulating microparticles, membrane vesicles bearing tissue factor derived from activated or apoptotic cells, are released into circulation and enhance fibrin formation.

Parallel to the increase in procoagulant activity, inflammation may impair fibrinolysis and affect anticoagulant factors. Fibrinolysis, a mechanism for clot resolution, is mediated by the enzyme plasmin derived from the proenzyme plasminogen. Inflammatory mediators, such as IL-1β and TNF-α, decrease synthesis of tissue-plasminogen activator and upregulate the production of plasminogen activator inhibitor (PAI)-1, which reduces plasmin levels. In human sepsis a significant elevation in the serum PAI-1 level was an effective predictor of mortality, supporting its potential as a biomarker in veterinary species. In sepsis and inflammatory conditions complicated by disseminated intravascular coagulation, levels of antithrombin III (ATIII), an inhibitor of thrombin and other coagulation factors, are reduced. Like PAI-1, ATIII levels are powerful prognostic indicators, with levels being significantly lower in nonsurvivors of sepsis in human beings. Sepsis also reduces ATIII levels in dogs; however, the levels do not necessarily correlate with survival. Another factor affected by inflammation, activated protein C, limits clotting by inactivating coagulation factors Va and Vila. Protein C becomes activated when thrombin binds to the receptor thrombomodulin on endothelial cells. Thrombomodulin expression is decreased by TNF-α, and thrombomodulin is inactivated by neutrophil-derived oxidants. Therefore inflammation decreases activated protein C levels, as reported in septic dogs. Therapy with recombinant human activated protein C preparations have shown antithrombotic effects in dogs; however, the large doses, short half-life, and cost of activated protein C have limited its use in veterinary medicine. Although recombinant human activated protein C (drotrecogin alfa) reached phase III clinical trials for sepsis treatment in human beings, it was removed from the market due to lack of beneficial effect in 28-day mortality.

**Kallikrein-Kinin System** The kallikrein-kinin system is composed of several classes of proteins: kallikrein proteases, kininogens, and kinins. Produced by the liver, kininogens are circulating plasma proteins such as high-molecular-weight kininogen and low-molecular-weight kininogen, which are cleaved by plasma or tissue kallikreins, a family of serine proteases, to form kinins. Kinins are vasodilatory proteins with...
local and systemic effects. They influence inflammatory responses, ischemia-reperfusion injury, and physiologic and pathophysiologic cardiovascular processes. The kinin system is intimately connected to the coagulation system and often is activated simultaneously. When prekallikrein and cofactor high-molecular-weight kininogen contact a negatively charged surface, factor XII is activated. Activated factor XII converts prekallikrein into the active kallikrein, which can then cleave kininogens. This further stimulates factor XII, causing a self-perpetuating enzymatic cascade producing kinins.

Bradykinin is the most notable of the kinins and interacts with two membrane receptors, B1 and B2. B2 receptors are ubiquitously and constitutively expressed in healthy tissue and are responsible for most of the basic homeostatic effects of bradykinin. In contrast, B1 receptors are produced de novo in many pathologic conditions. Although the mechanisms are not completely elucidated, the transcriptional factor nuclear factor kappa B (NFκB) plays an integral role in B1 receptor induction and regulation. Acting at these receptors, bradykinin is a potent mediator of endothelial prostacyclin synthesis, superoxide formation, and tissue plasminogen activator release. It also facilitates migration of polymorphonuclear leukocytes and stimulates cytokine synthesis. Similar to histamine, bradykinin stimulates venous dilation through local nitric oxide release, increases vascular permeability, and produces the associated pain response. These actions are ameliorated by the enzyme kininase, which quickly inactivates bradykinin.

Kinin deficiencies have been reported. Prekallikrein deficiency is actually the result of poor factor XII activation. The deficiency, characterized by a prolonged activated partial thromboplastin time (APTT) in the presence of normal coagulation factors, has been described in a dog, a family of miniature horses, and a family of Belgian horses. Although the resulting clotting abnormality is typically clinically insignificant, severe deficiency of this protein may complicate Surgical procedures.

**Tachykinins** A bidirectional pathway is present between the nervous and immune systems, facilitated by shared biochemical mediators (cytokines and neuropeptides) interacting with their respective receptors. Tachykinins are neuropeptides released from peripheral neurons after stimulation or direct trauma of sensory nerves. As part of the acute inflammatory response, histamine is one of the primary signaling molecules promoting tachykinin release. Substance P is a major tachykinin secreted by inflammatory leukocytes (macrophages, neutrophils, and eosinophils) and by prominent neurons in the respiratory tract, gastrointestinal tract, genitourinary system, central nervous system, and the dermal layer of the skin. Upon binding to G protein–coupled receptors designated as neurokinin receptors (NK-Rs), Substance P promotes transmission of pain and proinflammatory signals on effectors, including neurons, endothelial cells, and certain leukocytes. Substance P binding to NK1-Rs on neutrophils induces the release of prostaglandins such as COX-2 and PGE2. In endothelial cells, Substance P binds directly to NK1-R, initiating local vasodilation and increased venule permeability. Indirectly, Substance P also causes vasodilation and edema by promoting the synthesis of leukotrienes, nitric oxide, and prostaglandins. In addition to vascular effects, it stimulates leukocyte chemotaxis and leukocyte-endothelial cell adhesion, which collectively promote leukocyte extravasation. All of the cellular interactions resulting from Substance P stimulation may cause inflammatory signal amplification and enhanced nociception in affected tissue. Therefore an inflammatory response may alter neural function, and neuronal activity may modify immunologic function. With surgical trauma this is of particular importance because damaged nerves promote neurogenic inflammation and pain responses. Although there is some evidence that Substance P also functions in resolution of inflammation and tissue repair, the overall effects of Substance P appear to be proinflammatory; therefore direct inhibition of neuropeptides or upstream signaling molecules may have therapeutic potential for neurogenic inflammation.

**MEDIATORS AND OUTCOMES OF INFLAMMATION**

**Resolution** In the best-case scenario, acute inflammatory responses eliminate the inciting cause and lead to tissue repair. A fine system of checks and balances exists at every level of the inflammatory response, and many of those factors have already been discussed. Overall, resolution culminates with decreased polymorphonuclear neutrophil (PMN) recruitment and increased mononuclear cell infiltration. Inflammatory leukocytes are cleared through systemic recirculation and phagocytosis by macrophages. In turn, macrophages undergo apoptosis or exit inflamed tissues by lymphatic drainage. This process is modulated by a switch from proinflammatory to antiinflammatory factors, as well as toward “proresolution” factors, including IL-6, the CC chemokine CCL2, lipoxins, and many others. Uncomplicated resolution proceeds without inducing significant tissue damage. However, unchecked inflammation may have devastating consequences.

**Systemic Inflammation** The intensity of an inflammatory reaction is dependent upon the type and severity of the initial injury. It is also influenced by host factors (gender, age, genetics, nutrition), as well as by the presence of concurrent disease. When multiple factors coalesce, the intense inflammatory response may cause an imbalance of mediators, resulting in systemic manifestations. Even when the initial event is localized, soluble mediators may activate cells in organs distant to the inciting cause. In human beings this systemic inflammatory response syndrome (SIRS) is characterized by a well-defined set of parameters consisting of aberrations in body temperature, heart rate, blood pressure, respiration, and leukocyte counts. A diagnosis of sepsis is made when SIRS is accompanied by a nidus of infection. In veterinary medicine, SIRS is less well defined because of the extreme species variation in normal physiologic parameters and the fluctuation of these parameters caused by patient manipulation during measurement. Schemes based on parameters similar to those used in human beings have been described, particularly to aid the diagnosis of SIRS associated with infections such as pyometra and sepsis in dogs. However, it is important to remember that SIRS may be the result of many noninfectious causes, including neoplasia, pancreatitis, severe trauma, and autoimmune disease.

**Multiple Organ Failure** Multiple organ failure, also referred to as multiple organ dysfunction syndrome, is defined as the progressive dysfunction of two or more organ systems not involved in the initial physiologic insult. For example, patients admitted with burns, severe trauma, or sepsis actually may succumb to acute lung injury followed by liver, renal, and/or heart failure. It is postulated that multiple organ failure occurs secondary to major, self-destructive inflammation. Every major inflammatory mediator has been implicated in this process. Although the exact mechanisms are yet unknown, it is strongly believed that reperfusion-mediated oxidative injury to the gut epithelium serves as a major source of mediators driving the dysfunction...
of distant organs. Additional theories suggest that multiple organ failure results from a "two-hit" phenomenon in which the initial event primes neutrophils and macrophages for an exaggerated response to subsequent events such as secondary infection or prolonged hypotension. Ironically, these secondary, inflammation-inducing events include medical therapies such as surgical intervention and mechanical ventilation; therefore multiple organ failure is considered a disease of modern medicine, occurring in up to 50% of some human trauma patient populations. In veterinary medicine, similar technologic and therapeutic advancements allowing protracted patient survival will increase the occurrence of multiple organ failure.

**Immunosuppression**

Immunosuppression is an ironic complication of the inflammatory response. As mentioned earlier, the body has a number of means to counter the acute inflammatory response. Current theories suggest that SIRS and the compensatory antiinflammatory response essentially occur at the same time, with patient outcome dependent upon an appropriate balance of the two syndromes. An overwhelming antiinflammatory response can lead to immunosuppression, and an increased susceptibility to infection has been associated with sepsis, surgery, burn wounds, and trauma. Several mechanisms for the switch from proinflammatory to antiinflammatory conditions have been described earlier in this text. However, one of the most intriguing causes of postinflammation immunosuppression is endotoxin tolerance, which occurs when a cell exposed to endotoxin challenge becomes unresponsive to subsequent stimulation. Tolerance develops in many cell types but is most pronounced and well recognized in monocytes/macrophages. A number of mechanisms for tolerance have been described; however, recent gene analyses suggest that major "reprogramming" to downregulate inflammatory genes and upregulate antiinflammatory genes occurs when a cell is reexposed to endotoxin. A phenomenon called cross-tolerance may also occur, in which exposure to another stimulus, such as lipoteichoic acid from Gram-positive bacteria, leaves the cell unresponsive to endotoxin. Although these mechanisms may be protective adaptations to limit inflammation, the extreme manifestation of antiinflammatory responses can leave the host vulnerable to infection.

**Chronic Inflammation**

Prolongation of the inflammatory process is characterized by predominantly monocytic infiltrates, angiogenesis, and progressive tissue fibrosis. With persistent inflammation, inappropriate expression of proinflammatory factors by stromal cells leads to sustained recruitment, prolonged retention, and decreased apoptosis of inflammatory cells. In particular, fibroblasts play a significant role by expressing chemokines, including CXCL8/IL-8, CXCL12/stromal cell–derived factor 1 (SDF-1), and CCL2/monocyte chemoattractant protein (MCP)-1, which modulate the recruitment and functional behavior of inflammatory cells. In addition, fibroblasts express CD40 receptor, which, when engaged by its complementary ligand on activated T-cells, promotes expression of proinflammatory factors such as IL-6 and COX-2. This cross-talk between fibroblasts and inflammatory cells creates a cycle of persistent inflammation. In response to leukocyte-derived growth factors and cytokines, further differentiation of fibroblasts into myofibroblasts may occur; this increases production of extracellular matrix components such as collagen, which leads to tissue fibrosis that ultimately affects tissue strength and function. Granulomatous inflammation is a specialized type of chronic inflammation in which macrophages, epithelioid cells, and multinucleated giant cells aggregate into a well-demarcated lesion. Granulomas form in response to a variety of causative agents (e.g., foreign bodies, infectious agents, and toxins) and function to sequester the inciting agent. Sutures and starch from powdered surgical gloves have been reported as causes of postoperative granulomas and may incite fibrous adhesions in body cavities.

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See www.expertconsult.com for a complete list of references.
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