Innate Immunity in Critical Care

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Innate and adaptive immunity are required for effective control of infection. Numerous breakthroughs have been achieved in the last 15 years with regard to the functioning of the innate immune system. This article focuses on new paradigms of microorganism recognition, discusses recently described (or rediscovered) cytokines that provide further insight into the development of sepsis, and reviews both pro- and anti-inflammatory pathways for control of infection. Finally, it discusses what has and has not worked with regard to controlling inflammatory pathways in septic patients.

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The innate immune system has assumed increasing importance in the practice of critical care specialists because of its role not only in the clearance of microbes but also in tissue trauma, including direct injury, ischemia/reperfusion injury, and multiple organ system involvement. Traditionally, the immune system has been divided into innate and adaptive components. Clonal expansion of lymphocytes in response to infection is absolutely critical to the development of the immune response. However, 3 to 5 days are required for clonal expansion to produce sufficient numbers of “effector” cells. The innate immune system is fundamental in eliminating the infection or in controlling it until the adaptive immune response eliminates it. If the innate and adaptive immune responses are adequate, the infection remains localized. If not, then the systemic response to infection, or “sepsis,” ensues. Recent work has demonstrated that the adaptive response and innate immune system each can affect the functioning of the other.1

The hallmarks of inflammation, described by Cornelius Celsius (30BC to 38AD) as “rubror et tumor cum calore et dolore” (redness and swelling with heat and pain), are the interaction between microbes and essential elements of the innate immune system, the contact activation system, complement activation, and prostanoid production. Extensive studies conducted during the past 15 years demonstrate that inflammation and procoagulant host responses to infection are closely related. Inflammatory cytokines are capable of activating coagulation and inhibiting fibrinolysis, and thrombin is capable of stimulating multiple inflammatory pathways. The use of activated protein C in patients with severe sepsis has sparked a revolution in our understanding of how these two pathways converge in the intensive care unit (ICU).2 In this review, we focus on a few of these “new,” “re-discovered,” or perhaps “re-remembered” areas of innate immunity important to the critical care practitioner.

Pathogen-Associated Molecular Patterns

The innate immune system is phylogenetically ancient, and its recognition of infectious organisms is genetically pre-determined (i.e., has evolved by natural selection). Because microbes are heterogeneous and can mutate at very high rates, the innate immune system has developed receptors that recognize “pathogen-associated molecular patterns” (PAMPs), highly conserved structures present in a large group of microorganisms. PAMPs are produced only by microbial pathogens, not hosts; are structures recognized by the innate immune system and usually are essential for survival or pathogenicity of the microorganism; and usually are invariant structures shared by an entire class of pathogens. The best known examples of PAMPs are bacterial lipopolysaccharide (LPS), peptidoglycan, lipoteichoic acid (LTA), mannose, bacterial DNA, double-stranded RNA, and glucans.3

Pattern recognition receptors (PRRs) are the pathogen-recognition molecules of the innate immune system. They can be divided into three functional classes: secreted, endocytic, and signaling. Secreted PRRs, such as C-reactive protein (CRP), mannose-binding lectin (MBL), and others, bind to microbial cell walls and flag them for recognition by the complement system and by phagocytes, potentiating the phagocytosis of the bacteria. They also function indirectly by activating one of the complement pathways.4-6 Endocytic PRRs occur on phagocytes and mediate the uptake and delivery of pathogens into lysosomes, leading to microbial de-
Mannose Binding Lectin (MBL)

MBL and structurally related surfactant proteins A and D are members of the calcium-dependent lectin family that bind to microbial carbohydrates to initiate the lectin pathway of complement activation. MBL is synthesized in the liver and is secreted into the serum as an acute-phase response protein.\(^1,^4\) MBL is associated with serine proteases, known as MBL-associated proteases (MASP)-1 and -2, similar to C1r and C1s of the classic complement pathway (Fig. 1). Once activated, these proteases lead to the cleavage of the third component of complement and the activation of C3 convertase, which results in an amplified cascade of complement activation.

Toll-like Receptors (TLR)

Work by Janeway and Medzhitov revolutionized our understanding of the critical role of the innate immune system as the first step in adaptive immunity.\(^7\) They identified the human counterpart of a protein found in fruit flies (Drosophila melanogaster) known as Toll. The Toll protein in fruit flies is responsible for infectious susceptibility to fungi. Similar proteins are found in humans, the most studied to date being TLR-4. It is present on antigen-presenting cells (APCs), such as dendritic cells, macrophages, and monocytes, as well as other cell types, such as cardiac myocytes.\(^1,^8\) Human TLR-4 recognizes lipopolysaccharide. Through a complex signaling cascade requiring TLR-4 on the effector cell surface, along with plasma factors LPS-binding protein (LBP), CD14 (present in plasma and/or on the cell surface), and MD-2, LPS stimulation results ultimately in the activation of cytoplasmic nuclear factor-\(\kappa\)B (NF-\(\kappa\)B). Through this pathway, the activation of TLR-4 induces the expression of a variety of cytokines and costimulatory molecules that are crucial to both the innate and adaptive immune responses. The members of the TLR family (11 identified so far in humans) recognize pathogens from protozoa, bacteria, fungi, and viruses.\(^0,^10\) All of these TLRs signal through a number of adapters and protein kinases that are linked to downstream signaling events, resulting in gene transcription through “proinflammatory” transcription factors, NF-\(\kappa\)B, activator protein-1 (AP-1), and signal transducer and activator of transcription-1 (STAT1).\(^9\) These factors result in transcription of many genes, including tumor necrosis factor (TNF) and interferon-\(\beta\) (IFN-\(\beta\), as well as T-cell costimulatory molecules. Whereas TNF is a central mediator in the innate immune response, IFN-\(\beta\) is a critical factor in adaptive and antiviral immunity. Presentation of antigen by the major histocompatibility molecules II (MHCII) on the APC is insufficient to induce the activation of the T-cell receptors. Activation of the T cell through the T-cell receptor also requires the expression of CD80 or CD86 on the APC. Thus, TLRs are critical proteins linking innate and acquired immunity.\(^10\)
Endogenous Antimicrobial Peptides

A number of peptides are secreted or present on the epithelial surface of the skin, gastrointestinal tract, and bronchial tree and have endogenous antimicrobial activity. Not surprisingly, these same or similar peptides are found in the leukocytes. These proteins include the defensins, cathelicidin, lactoferrin, and bacterial permeability increasing (BPI) factor. These antimicrobial peptides are heavily positively charged. Acting as PRRs they specifically target bacterial cell membranes for which the outermost leaflets of the lipid bilayer are populated with negatively charged phospholipids. The means by which they actually “kill” microbes remains unclear, but it could be their ability create physical holes from which the cell contents leak. BPI deserves special mention. It was identified initially in the primary granule of neutrophils. Because of its high affinity for LPS, BPI is particularly cytotoxic for gram-negative bacteria. It also can function as an opsonin-enhancing phagocytosis. Recombinant BPI was cytotoxic for gram-negative bacteria. It also can function as an opsonin, which the cell contents leak. BPI deserves special mention. It was identified initially in the primary granule of neutrophils. Because of its high affinity for LPS, BPI is particularly cytotoxic for gram-negative bacteria. It also can function as an opsonin-enhancing phagocytosis. Recombinant BPI was tested in trials performed on 400 children with severe meningococcal sepsis. Although BPI had no effect on survival, fewer patients required multiple severe amputations, and by day 60 those who had received BPI had more functional outcomes than did those patients who had not received BPI.

Complement System

The complement system is positioned critically to participate in both the innate and the adaptive immune responses. For greater than 100 years, the complement system has been known to protect the body from pathogenic organisms. It is critical to highlight that the complement system transmits both stimulatory and inhibitory signals to many cell types, leading to cell activation, division or proliferation, cell movement, adherence or secretion, and cell death or rescue from death. The activation of the classical pathway of complement through antibody-microbial interactions resulting ultimately in microbial lysis can occur only through the adaptive immune system. However, complement activation can be initiated through the classical pathway by apoptotic cells, certain viruses and gram-negative bacteria, and CRP bound to ligand (ie, no antibody is needed) (Fig. 1). Complement also can be activated through the alternative and the recently described MBL pathways. The alternative pathway is initiated directly by many bacteria, fungi, viruses, and tumor cells. As outlined previously, the MBL pathway is activated by microbes with terminal mannose groups to which the acute-phase protein MBL will bind.

The complement system amplifies the initial response to the organism. In addition, the regulatory mechanisms of complement are finely balanced such that the activation of complement is focused on the surface of the invading microorganisms and the deposition of complement on normal cells is limited. Although complement activation can occur through one of the three pathways (Fig. 1), all three converge at the cleavage of C3. Cleavage of C3 results in the release of the anaphylatoxin C3a and formation of C3b, which can bind to the microbial surface, acting as an opsonin. C3b also assists in the formation of the C5 convertase, as it “accepts” C5. C5 cleavage results in the formation of the anaphylatoxin C5a and the fragment C5b, which initiates the formation of the membrane-attack complex. The membrane-attack complex inserts into the cell membrane, creating large pores, thereby leading to osmotic lysis of targets.

In addition to lysing the target organisms, opsonization of target cell membranes with complement fragments C3b/C4b and C3bi (an inactive fragment of C3b) results in phagocytosis by neutrophils, monocytes, and macrophages. Binding occurs through specific complement receptors, CR1 (CD35) and CR3 (CD11b/CD18, Mac-1), respectively. Also critical in complement activation is the formation of the anaphylatoxins C3a, C4a, and C5a, which are responsible in part for edema and increased vascular permeability that occurs with complement activation. The latter occurs through the release of histamine from mast cells and through the local production of vasodilatory prostaglandins such as prostaglandin E2, and the edemogenic leukotrienes (LT) C4, D4, E4.

C5a has a strong chemotactic effect on neutrophils, but it also can induce the release of granular enzymes from phagocytic cells and the production of superoxide anion. The action of C5a occurs through its seven transmembrane-spanning, G-protein coupled receptor, C5aR. In addition to being present on leukocytes, C5aR is found on other tissue, including brain, kidney, liver, and gastrointestinal tract, clearly indicating its role as a central modulator in many inflammatory disease states, including ischemia/reperfusion. Blocking the function of C5a improves survival rates and lessens the development of multiple system organ failure in mice models with abdominal sepsis (cecal ligation puncture). In addition, the systemic effects on neutrophil activation that occur with sepsis are reversed.

For more than 30 years, complement activation has been known to occur early after an ischemia/reperfusion injury. The use of strategies to block complement activation in animal models of ischemia/reperfusion has been successful, but translating this use into successful trials in humans has been slow. However, the use of a humanized monoclonal antibody fragment, pexelizumab, that binds specifically to C5, preventing its cleavage and ultimately terminal component activation in patients with myocardial ischemia, has provided a basis for ongoing studies. In those patients undergoing percutaneous coronary intervention with acute myocardial infarction who received a bolus dose plus 24-hour infusion of pexelizumab (ie, complement inhibition for 24 hours), the 90-day mortality rate was reduced compared to that of those who did not receive it (5.9% to 1.9%, p = 0.014), although no difference occurred in infarct size. In patients undergoing elective coronary bypass, pexelizumab decreased the composite endpoints of death and myocardial infarction at day 30. In neither study was there evidence of an increased incidence of infections nor of impaired healing. Important to highlight in these trials is that a reduction in the mortality rate without a reduction in the infarct size suggests that pexelizumab might be modulating the subacute inflammatory consequences of ischemia and/or infarct healing. The use of
pexeluzimab in patients with sepsis would be problematic at best because of the critical role of complement in both the innate and acquired immune responses in the clearance of bacteria. Animals (and humans) with complement deficiency are at increased risk of developing sepsis. However, therapies that inhibit the effects of C3a may be of benefit.

**Contact Activation System and Arachidonic Acid Metabolites**

Four major plasma protein systems contribute to the host’s defense and participate in the development of inflammatory tissue injury. In addition to the complement system, the extrinsic coagulation, and fibrinolytic system, is the contact activation system (also known as Hageman factor or intrinsic coagulation system). The contact activation system is critical to host defense and control of local blood flow at sites of injury. Hageman factor (Factor XII) is activated (XIIa) spontaneously on contact with negatively charged surfaces such as lipopolysaccharide or extrinsic agents such as plastic tubing/membranes. HMWK–prekallikrein–factor IX are complexed in the plasma. Factor IXa that is formed can amplify the extrinsic coagulation cascade. Bradykinin production drives the production of arachidonic acid and PAF by upregulating phospholipase A2 activity. Arachidonic acid is the precursor for prostaglandins, leukotrienes, and other prostanoids. PAF, platelet activating factor; LMWK, low molecular weight kininogen; HMWK, high molecular weight kininogen.

**Bradykinin**

Bradykinin is an exceedingly potent vasoactive peptide. It can cause venular dilation, increased vascular permeability, hypotension, bronchoconstriction, and activation of phospholipase A2. Bradykinin is metabolized by angiotensin-converting enzyme (ACE) to inactive peptide. Phospholipase A2 activation results in the production of arachidonic acid, a 20 carbon fatty acid from cell membrane phospholipids, and a series of compounds known as platelet activating factors. Production of arachidonic acid and its prostanoid metabolites (prostaglandins and thromboxanes) via cyclooxygenase (COX)-1 and COX-2, along with bradykinin, enhances pain sensation and formation of exudate in inflammatory tissues. Animal models have shown that bradykinin is responsible for the four signs of inflammation first identified by Celsus: heat, redness, swelling, and pain. Bradykinin along with prostanoid stimulates the pain response in mammals through polymodal receptors and capsaicin-sensitive fibers (C-fibers). The bradykinin effect is enhanced by simultaneous production of prostanoids. The prostanoids, along with the low pH of exudates, inhibit the activity of kininases such as ACE.

More than 20 years ago, hypotension with vasodilation and erythema was observed to be a transfusion reaction associated with the rapid infusion of albumin and plasma protein fraction. These reactions were even more prevalent and severe in patients undergoing cardiopulmonary bypass. At least a portion of these reactions appeared to be caused by the production of bradykinin. These effects were potentiated in patients receiving ACE inhibitors. Such transfusion reactions have been minimized by altering exposure to various plastics, avoiding the use of ACE inhibitors, and minimizing the use of white blood cell (WBC) and platelet removal filters at the bedside.
also induces numerous leukocyte responses, including gene expression, directional migration, and generation of oxygen radicals. The presentation of PAF along with adhesion molecules on endothelial surfaces specifically recognized by leukocytes results in juxtacrine signaling of the leukocyte facilitating the transition of a rapidly translocating white blood cell to one that is arrested.

In the 1980s, PAF was noted to be elevated in adults and children with sepsis. PAF acetylhydrolases are enzymes that recognize PAF and cleave PAF to yield products that no longer are recognized by the PAF receptor. The plasma or secreted form of PAF acetylhydrolase is constitutively present in plasma and is produced by macrophages. Studies demonstrate that PAF acetylhydrolase is diminished or inactive in patients with sepsis. Prolonged clearance of PAF occurs in patients with sepsis. Although improved survival was noted in a phase II trial of septic patients treated with recombinant PAF acetylhydrolase, a large multicenter trial did not demonstrate a benefit.

Cytokines, Chemokines, and Modulators of the Immune Response

Cytokines are signaling proteins secreted by cells that affect the functional properties of other cells of the same organism. The cytokine family includes the lymphokines, chemokines, interleukins, and interferons. Cytokines may travel short or long extracellular distances before interacting with target cell surface receptors in a paracrine, autocrine, or endocrine manner. They can be detected in serum samples, particularly during times of maximal production, as occurs in sepsis. Cytokines as a group are low-molecular-weight proteins of less than 80 kilodaltons. They interact with high-affinity cell surface receptors specific for each cytokine. Their cell binding ultimately leads to changes in the pattern of protein synthesis, altered cell behavior, or both. They often have multiple overlapping regulatory functions. Cytokines that are considered “pro” inflammatory include TNF-α, IL-1β, IL-18, and IFN-γ. IL-10 and IL-6 are pleiotropic cytokines with both pro- and anti-inflammatory effects. Chemokines are small proteins (8–14 kDa) with the primary function of regulating cell trafficking. IL-8 (CXCL8) and MCP-1 (monocyte chemotactic peptide-1, CCL2) are two of more than 50 chemokines identified to date.

IL-1 and TNF
IL-1 is a phylogenetically ancient molecule that predates the evolution of lymphocytes and immunoglobulin. Its activity extends beyond immune function. It is produced by a wide variety of cells, including macrophages, endothelial cells, epithelial cells, and vascular smooth muscle cells. TNF is produced by cells primarly of the innate immune system, including monocytes/macrophages, natural killer (NK) cells, mast cells, and neutrophils. TNF is produced by other cell types under specific stress conditions, for example, the cardiac myocytes in congestive heart failure and in sepsis. Both cytokines are “pro-inflammatory” and both have two forms. Signaling of cells by TNF-α and IL-1β occurs in part through NF-κB and, consequently, they share a similarity in receptor function and signaling molecules. Both IL-1β and TNF are expressed and secreted transiently and are early mediators of septic shock. Their activities are modulated by co-production of naturally occurring anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1 RA). With the background of promising animal studies and small phase II trials in humans, multiple trials using immunomodulator therapies against either TNF or IL-1 have been performed in adults. In two trials enrolling more than 2500 patients, recombinant IL-1 RA was no more effective than was placebo in treating patients with sepsis or severe sepsis. A series of Phase III trials of more than 3500 patients have used anti-TNF strategies but none has demonstrated improved outcome in patients with sepsis.

High Mobility Group-1B

The observations that have led to the search for late mediators of sepsis are (1) TNF-α and IL-1β reach toxic levels in mice and human volunteers 1 to 2 hours after infusion of LPS; (2) TNF-α and IL-1β levels are not elevated in patients with sepsis; (3) treatment of animals with anti-TNF or anti-IL-1 failed to prevent late endotoxin deaths; and (4) endotoxin-induced death in mice occurred up to 5 days after infusion, long after serum TNF-α and IL-1β returned to baseline values. One of these new mediators is high mobility group 1 B (HMGB1). Tracey and colleagues stimulated murine macrophage-like cells with LPS and screened conditioned media for proteins appearing 16 hours after stimulation. They identified a 30-kDa protein identical to HMGB1, a member of the high mobility group 1 nonhistone chromosomal protein family. Mice showed increased serum levels of HMGB1 8 to 32 hours after exposure to endotoxin. In addition, delayed administration of antibodies to HMGB1 attenuated endotoxin lethality, and administration of HMGB1 itself was lethal. HMGB1 now is known to be secreted by activated macrophages, mature dendritic cells, and NK cells in response to injury, infection, or other inflammatory stimulus. HMGB1 transduces cellular signals through RAGE (receptor for advanced glycation end-products) and likely through TLR2 and TLR4 as well as other receptors. HMGB1 appears to be actively secreted by monocytes/macrophages through a process of acetylation and and migration to cytoplasmic secretory vesicles. HMGB1 can be released by necrotic cells. HBGB1 normally is bound loosely to chromatin; therefore, if a cell becomes necrotic or “leaky,” then HMGB1 can diffuse out into the extracellular space. In contrast, when a cell dies by apoptosis, HMGB1 binds tightly to cruciform DNA, and release of HMGB1 is inhibited. This mechanism may explain in part why death of cells by necrosis results in inflammation, whereas death by necrosis does not. Note that in neutrophils, HMGB1 is tightly sequestered to the cell membrane, which limits its release even under conditions of necrotic cell death. HMGB1 promotes the recruitment of leukocytes across endothelial barriers through the effects on integrin signaling.
HMGB1 interacts with RAGE expressed on endothelial cells, inducing endothelial activation, with increased expression of vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial cell selectin (E-selectin). Thus, activation of the endothelium through HMGB1/RAGE can result in increased localization of leukocytes to the inflamed endothelia. HMGB1 has effects on the lung, liver, gut, and the central nervous system in processes ranging from hemorrhagic shock to Alzheimer disease to arthritis.36

Macrophage Migration Inhibitor Factor (MIF)
Macrophage migration inhibitor factor (MIF) was one of the first cytokines identified almost 40 years ago. Molecular cloning of MIF led to the rediscovery of MIF as a molecule released by cells of the anterior pituitary after exposure to the LPS. Despite its cytokine activities, MIF does not seem to belong to any cytokine superfamily. All mammalian MIFs have approximately 90 percent homology. MIF is expressed constitutively by immune and endocrine cells and also by the epithelial lining of tissues in direct contact with the external environment. Microbial products and proinflammatory cytokines also induce the release of preformed MIF. Targeted disruption of MIF in mice resulted in protection against LPS and Staphylococcus aureus enterotoxin B administered systemically, and improved clearance of pseudomonas aeruginosa administered via the trachea.37 Elevated MIF levels were found in patients with sepsis and septic shock, and elevated levels had increased discriminatory power to predict mortality compared with IL-6.38 Elevated intracellular amounts of MIF were found in T cells, B cells, and monocytes of patients with severe sepsis compared with healthy control patients.39 MIF is recognized as a potent suppressor of the anti-inflammatory effects of glucocorticoids and, thus, may serve this role in vivo.40,41 In neonates with necrotizing enterocolitis, MIF was observed in the infiltrating macrophages in the mucosa and submucosa layer of the affected gut, and serum levels of MIF, IL-6, and IL-8 were elevated during the acute stage of the disease.42 MIF has been hypothesized to be the “control” point of vascular hyporesponsiveness in septic shock.40 Recent data suggest that MIF may have “chaperone”-like activity, as seen with heat shock proteins and HMGB1, which would explain its pleiotropic effects.

Nitric Oxide
Nitric oxide is a stable, free radical gas. Extensive work performed during the past 20 years has converged to establish nitric oxide as a major messenger regulating immune function and blood vessel dilation and serving as a neurotransmitter. In intensive care units, (ICUs) nitric oxide has joined the armamentarium for the control of pulmonary blood vessel tone. However, nitric oxide is critical for immune functioning, particularly in adaptive immunity to intracellular pathogens such as Mycobacterium tuberculosis and Listeria monocytogenes. Nitric oxide, which is produced by nitric oxide synthase from the metabolism of arginine, enhances the bactericidal activity of NK cells and macrophages. The interaction of intracellular nitric oxide and reactive oxygen products results in the production of peroxynitrite, which contributes in part to the killing of the microorganism. Nitric oxide also is produced in the epithelial cells lining the nasal sinuses, resulting in very high concentrations.43 These elevated concentrations are hypothesized to be required for maintaining sterility of the sinuses.43 In patients with sepsis and maxillary sinusitis by radiograph (not by culture), nitric oxide synthase is reduced and airway nitric oxide levels are subsequently lower.44 Because patients with sinusitis in the ICU are known to have increased risk of acquiring nosocomial pneumonia and because aggressive treatment of sinusitis decreases pneumonia risk, it has been suggested that drainage of maxillary sinusitis, even when the suspicion of infective sinusitis is low, would result in improved nitric oxide synthase production and protect the patient from nosocomial infections.44

The Neuroimmune Axis
For decades, evidence has shown the possible existence of direct links between the immune and the nervous systems. Clearly, immune cells can produce peptide hormones and neurotransmitters; virtually all neuropeptide, neurotransmitter, and neuroendocrine hormone receptors also are present on cells of the immune system; and neurons can make cytokines such as IL-1.45 Thus, in the afferent limb of the neuroimmune axis, cytokines such as IL-1 can act on the vagus nerve to cause behavioral changes and symptoms of illness. Lymphocyte-derived neuropeptides such as β-endorphin may modulate pain sensations by acting on peripheral sensory nerves. IL-1 derived from macrophages can act on the hypothalamus and pituitary to produce corticotrophin-releasing hormone and ACTH, respectively. Leukocyte-derived hormones such as α-melanocyte-stimulating hormone can cross the blood-brain barrier and directly signal the sympathetic nervous system. Thus, Blalock has described the immune system as a “sixth sense” that provides inflammatory messages to the nervous system, much as other senses provide neural input.45

Intriguing data have been reported recently regarding the efferent limb of the neuroendocrine axis. The cholinergic nervous system appears to function as the pathway that reflexively monitors and modifies the inflammatory response.46 In rats challenged with endotoxin, surgical transection of the vagus nerve led to enhanced systemic TNF-α production and accelerated the development of shock.47 Electrical stimulation of the vagus nerve down-regulated TNF-α production and improved survival in these rats. The vagus nerve exerts the anti-inflammatory effects through its major neurotransmitter acetylcholine, which interacts with nicotinic acetylcholine receptors on macrophages.47,48 Note that the macrophages, and not monocytes, are responsive to acetylcholine.47 In mice with septic peritonitis from intraperitoneal injection of Escherichia coli, initial release of cytokine was enhanced, cellular infiltrate into the peritoneum was greater, and liver injury was potentiated if animals previously had a
vagotomy compared to sham animals. In contrast, if animals had been pretreated with nicotine, then subjected to peritonitis, they had a diminished number of leukocytes in the peritoneum, decreased proinflammatory cytokine production, and attenuated liver injury compared to control animals. Although vagotomy had no effect on the clearance of bacteria from the blood, peritoneum, or liver and did not affect survival, pretreatment with nicotine decreased the clearance of bacteria and shortened the survival time. The effects of acetylcholine also appear to be due directly to receptors present on endothelial cells, as cholinergic agonists inhibited TNF-induced endothelial activation by blocking NF-κB entry into the nucleus. Critically ill patients appear to have an uncontrolled increase in “anti-inflammatory” responses that is associated with multiple-system organ failure and death. Autonomic dysfunction is associated with increased risk of developing sepsis and death in patients in the ICU. Thus, the loss of autonomic function in these patients possibly results ultimately in patients who are “immunologically suppressed” due to increases in the cholinergic anti-inflammatory pathway.

**Cellular Components of Innate Immunity**

The cellular components of immunity traditionally have been divided into innate and adaptive immunity, but such distinctions have become increasingly blurred, and critical overlap occurs. For example, dendritic cells and other antigen presenting cells, such as macrophages and monocytes, are part of the innate immune system, although they directly drive adaptive immunity.

**Natural Killer Cells**

Natural Killer (NK) cells are large granular lymphocytes that have cytotoxic function and the ability to produce cytokines and chemokines. NK cells are critical for the early control of viral infections until peptide-specific T lymphocytes can be generated. NK cells are functionally distinct from cytotoxic T lymphocytes because they can lyse virally infected cells in a non-MHC manner, thus not requiring previous sensitization to that virus. They also can kill microbes in an antibody-dependent manner. As such, these cells are part of the innate immune system. Production of IFN-γ by activated NK cells potentiates the macrophage/monocyte/dendritic cell for TNF production and release of factors (IL-1, IL-18, IL-12) that can feedback and further stimulate the NK cell. IFN-γ produced by NK cells also leads to activation of T cells. Activated NK cells are the main source of IFN-γ during sepsis. Depletion of NK cells in septic mice offers protection against cytokine and LPS-induced shock. In patients with septic shock, those with evidence of increased cytotoxic cell function had a higher mortality rate and worse organ function.

**Platelets**

Platelets usually are not considered part of the innate immune response. But as noted throughout this monograph, hemostasis and inflammation overlap. The endothelial surface, which usually is “anticoagulant,” becomes “procoagulant” with infection and inflammation. Platelets themselves have inflammatory, antimicrobial and immune modulating effects. In a platelet thrombus, neutrophils and, to a lesser extent, monocytes are recruited to the developing thrombus via adhesion receptors present on the platelets themselves. Platelets secrete numerous stored chemokines that activate neutrophils, monocytes, T cells, and NK cells. Platelets contain anti-microbial peptides. They can synthesize IL-1β, and they can release TNF from pre-formed pools. Platelets can interact with neutrophils to produce pro- and anti-inflammatory products from arachidonic acid metabolism.

**Phagocytes**

Phagocytes include neutrophils, eosinophils, monocytes/macrophages, and dendritic cells. They have in common numerous different properties that are critical to host defense and the inflammatory response. These cells share the ability to phagocyte foreign material and secrete inflammatory mediators and regulators. All but the dendritic cells release granule constituents into phagolysosomes and form reactive oxygen products through a unique reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme system. Neutrophils, eosinophils, and monocytes share similar mechanisms of cell migration, phagocytosis, and pathogen killing. Neutrophils are the main defense against bacterial and fungal infections, whereas eosinophils are important in the control of parasitic infections. Neutrophils remain in the storage pool of the bone marrow for as long as 5 days. They are released from the marrow into the bloodstream, with half of them actively circulating for approximately 10 hours and the others remaining in the marginalized pool. The marginalized pool can be mobilized in response to stress, infection and inflammation. Once neutrophils migrate into the tissue, they survive for 1 to 2 days. However, their survival in inflammatory foci is prolonged as a result of the presence of growth factors such as granulocyte- or granulocyte/macrophage-colony stimulating factor (G- or GM-CSF). These growth factors were identified initially by their ability to induce granulocytopoiesis and monocytopenia; however, they have marked effects on neutrophils and monocyte function. Their production occurs outside the bone marrow stromal cells. They are produced by activated monocytes/macrophages and fibroblasts. G-CSF also is produced by epithelial cells of the gut and lung and by neutrophils themselves. GM-CSF is produced by T lymphocytes and NK cells. Apoptosis is delayed in the presence of G- or GM-CSF, and removal of these cytokines, as occurs in the resolution phase of inflammation, leads to induction of apoptosis and increased clearance of neutrophils.

**Monocytes and Macrophages.**

Monocytes and macrophages are part of the mononuclear phagocyte system that also includes dendritic cells. Although these cells share characteristics with neutrophils and eosinophils, they also have unique properties including antigen
processing and interaction with lymphocytes in the generation of immune response and the clearance of apoptotic cells (a property specifically of macrophages). Monocytes emigrate into tissue both as part of the immune response and as replacements for macrophages. Macrophages are found in pleural, synovial, and peritoneal space/cavity and in the alveoli. Fixed macrophages are less motile and include those in the splenic sinusoids, liver (Kupffer cells), bone marrow, lamina propria of the gastrointestinal tract, and the lymph node reticulum, but also as osteoclasts and as microglia.

Once the leukocyte arrives at the site of bacterial/pathogen invasion, receptors are activated, and the cell membrane surrounds and engulfs the pathogen. However, the membrane advances only the portion of the particle or pathogen that is “opsonized” or where the “molecular pattern” fits the appropriate receptor on the phagocyte. In addition to complement, scavenger, and mannose receptors on the leukocyte surface are receptors that recognize the Fc portion of immunoglobulin. Neutrophils and monocytes have granules that contain microbial enzymes, myeloperoxidase, proteases, cationic proteins, BPI, and defensins. Once the phagolysosome is formed, granule contents are released into it. Also in the phagolysosome there is the production of superoxide followed by other oxygen metabolites (i.e., hydrogen peroxide and hypochlorous acid). Thus, the combined effects of antibacterial agents and oxidants result in microbial killing. Individuals with chronic granulomatous disease have a genetic defect in respiratory burst activity. They are unable to form oxidants derived from respiratory burst, and their absence results in recurrent, often life-threatening, bacterial and fungal infections.

Leukocyte Localization

Leukocyte localization to a site of infection is a multiple-step process that follows the following paradigm 1 process (Fig. 3). The interested reader is referred to extensive reviews for more details. The focus here is on the molecular events with regard to neutrophils. The process begins with the activation of the post-capillary venular endothelial surface by inflammatory cytokines such as IL-1β, TNF-α, and IFN-γ (Fig. 3). The endothelial surface transforms from a nonadhesive surface to one that is proadhesive through expression of specific ligands. These endothelial ligands (E-selectin [CD62-E] and P-selectin [CD62-P] and other selectin ligands) recognize receptors on the leukocyte surface (P-selectin glycoprotein ligand-1 [CD162] and leukocyte selectin [L-selectin CD62-L]). This process results in the “capture” of the leukocyte from the free-flowing stream. The tethered cell then may “roll” on the endothelial surface for a short distance and either transition to an arrested cell or return to the free stream. The transition from a rolling to an arrested cell is dependent on “activating” substances such as chemokines and PAF. Leukocytes are captured from the free-flowing stream (tether) and roll on the endothelial lining of the blood vessel. This interaction is dependant on endothelial selectins (E- and P-selectin), and receptors on the leukocyte (L-selectin, and PSGL-1). In order for leukocytes to transition to an arrested cell, they must be activated. This occurs via chemokines/PAF presented on the endothelial surface and the process of rolling itself. This cell signaling results in activation of the leukocyte integrins, LFA-1, Mac-1, p150,95, and VLA-4. These ligands and JAM-1 interact with members of the immunoglobulin gene superfamily, ICAM-1 and VCAM-1 present on the endothelial surface. Transendothelial migration occurs and the leukocyte then must navigate the subendothelial basement membrane and connective tissue. Platelets may attach directly to inflamed endothelium or to denuded basement membrane through vWF. Platelets can serve as a surface to which leukocytes can tether directly. Leukocytes can bind to platelet P-selectin, platelet GPIb-IX, or through fibrinogen attached to platelets. Platelets release chemokines that can activate leukocytes. Leukocytes also can attract other leukocytes through L-selectin-PSGL-1 interactions. PSGL-1, P-selectin glycoprotein ligand-1, PAF, platelet activating factor, vWF, von Willebrand factor, Ig, immunoglobulin.

Figure 3 Leukocyte localization. The endothelial surface is activated by inflammatory cytokines to produce the selectins, Ig Superfamily adhesive molecules, and activating substances such as chemokines and PAF. Leukocytes are captured from the free-flowing stream (tether) and roll on the endothelial lining of the blood vessel. This interaction is dependant on endothelial selectins (E- and P-selectin), and receptors on the leukocyte (L-selectin, and PSGL-1). In order for leukocytes to transition to an arrested cell, they must be activated. This occurs via chemokines/PAF presented on the endothelial surface and the process of rolling itself. This cell signaling results in activation of the leukocyte integrins, LFA-1, Mac-1, p150,95, and VLA-4. These ligands and JAM-1 interact with members of the immunoglobulin gene superfamily, ICAM-1 and VCAM-1 present on the endothelial surface. Transendothelial migration occurs and the leukocyte then must navigate the subendothelial basement membrane and connective tissue. Platelets may attach directly to inflamed endothelium or to denuded basement membrane through vWF. Platelets can serve as a surface to which leukocytes can tether directly. Leukocytes can bind to platelet P-selectin, platelet GPIb-IX, or through fibrinogen attached to platelets. Platelets release chemokines that can activate leukocytes. Leukocytes also can attract other leukocytes through L-selectin-PSGL-1 interactions. PSGL-1, P-selectin glycoprotein ligand-1, PAF, platelet activating factor, vWF, von Willebrand factor, Ig, immunoglobulin.

The leukocyte then must migrate between the endothelial cells and negotiate the basement membrane and subbasement membrane, which is composed of collagen, vimentin, laminin, and fibronectin, to arrive at the site of infection. Adhesion and locomotion on basement membrane proteins are dependent on leukocyte β1 integrins (very late antigen [VLA]1-6. Important to highlight are: (1) all leukocytes use a similar multiple-step process for localization whether it be to an inflammatory focus or part of normal immune surveillance; (2) for leukocyte localization in organs such as the lung, brain, liver and kidney, the mechanisms may be similar, but localization also may be caused by physical trapping in specialized capillary/sinusoidal regions; (3) adhesion molecules normally found on leukocytes, platelets, or endothelial cells often are found in the circulation and have been used as...
markers of inflammation and the blockade of adhesion molecule function has been attempted in numerous disease states with variable success. Specifically, trials in traumatic shock, stroke, ischemia-reperfusion injury, myocardial infarction, and burns have not been successful, whereas those for inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, and psoriasis have. In several genetic defects, adhesion molecule expression is either absent or malfunctioning. These conditions include leukocyte adhesion deficiency 1 (LAD 1), in which the leukocyte $\beta_2$ integrins are affected, and LAD 2, in which the leukocyte selectin molecules are nonfunctional. Both groups of patients have extreme neutrophilia with severe recurrent skin abscesses and life-threatening infections.

**Host Critical Illness Interaction**

**Effects of Critical Illness on Leukocyte Function**

Neutrophil function is not “normal” in patients with critical illness, which may contribute in part to the high incidence of nosocomial infections. Neutrophils from traumatized or septic patients demonstrate delays in chemotaxis both in vivo and in vitro, and these delays were associated with increased rates of infection risk. Whereas some studies have identified changes in expression of adhesion molecules, others have not. Several studies demonstrate that oxidative burst remains intact or increased and may be associated with outcome. Those neutrophils with the highest oxidative burst and those with less granule content appear to become trapped in the lung, providing some insight into acute lung injury associated with sepsis. Finally, apoptosis in critically ill patients is profoundly suppressed compared to that in those who are not.

What has become clear during the past 2 decades is that during acute infection, the body produces both pro- and anti-inflammatory responses, but the anti-inflammatory response quickly predominates. This sustained immunosuppressive profile is called immunoparalysis and is characterized primarily by T-cell anergy and a defect in antigen presentation. The decreased expression of human leukocyte antigen DR (HLA-DR) on monocytes constitutes a reliable marker of immunoparalysis and seems to be associated with risk of fatal outcome and to be a predictor of sepsis in adults and children. Associated diminished LPS-stimulated TNF- production in monocytes also occurs, although G-CSF production is augmented.

**Genetic Polymorphisms in Sepsis**

The response to infection is variable among individuals. Given the same therapies, most patients will do well, although a small but significant proportion will develop sepsis, multiple-system organ failure, and death. What is becoming increasingly clear is that these different responses appear to be due to the genetic makeup of the host. An early study supporting this hypothesis by Sorensen and colleagues revealed that if a biologic parent died before the age of 50 from infection, his or her child had a 4.52 relative risk of also dying from an infection. This effect was not seen in adopted children and their adoptive parents. Since this landmark study, more than 200 articles have been published addressing this association. Sequencing of the human genome has demonstrated that many genes are polymorphic. A polymorphic gene is one in which a comparison of the DNA sequence in multiple individuals shows differences at a frequency of greater than 1 percent. The sites that are different are called polymorphic sites, and they may differ by insertions, deletions, or substitutions of one or more base pairs or by the presence of a variable number of repeats of short, repetitive DNA sequences. Some of these variations have been shown to influence the level and/or activity of the resulting protein. Polymorphisms important to the innate immune response include those proteins critical for immune recognition (e.g., TLR4, CD14, Fc receptors, mannose-binding lectin) and those for immune response (e.g., TNF-$\alpha$, IL-1, IL-1RA, IL-6, IL-10).

**Immunomodulation and Hemostasis in Sepsis**

As discussed previously, therapies used to counteract pro-inflammatory mediators such as TNF-$\alpha$, IL-1$\beta$, and others and anti-adhesive therapy do not affect the outcome of sepsis and septic shock. Attempts at immunomodulation have been equally disappointing. IFN-$\gamma$ has not been shown to be of benefit in patients with trauma and burns, and variable therapeutic efficacy has been achieved in the clinical trials of G-CSF and GM-CSF to date. A systematic review examined the efficacy and safety of therapy with G-CSF or GM-CSF in almost 600 newborn infants. At this time, evidence is insufficient to support the introduction of either G-CSF or GM-CSF into the care of the neonate either as a treatment for established infection or as prophylaxis to prevent systemic infection. Nonetheless there are critical differences in the function of G-CSF and GM-CSF. G-CSF’s effects are primarily on mature neutrophils, whereas GM-CSF has a pleiotropic role. In a recent study of septic adult patients, more than half of whom had an organ transplant, the use of GM-CSF significantly increased the number of patients with a cure or improvement of their primary infection, although there was no statistical effect on outcome. Although it is too early to support the use of this agent routinely, this finding does highlight that specific agents may be helpful, but population selection is critical, and likely as to why immunomodulatory therapy has not been successful in these conditions.

To date, only one drug has affected significantly the outcome in adult patients with sepsis. Surprisingly, that drug is the activated form of the naturally occurring anticoagulant protein C (drotrecogen alfa [activated], Xigris). Activated protein C decreased death rates by 20 percent in the PROWESS trial of 1690 patients. Data suggest that the value of activated protein C may reside less in its role as an anti-thrombotic and more in its role as an anti-inflammatory by inhibiting neutrophil activation, production of cytokines, and leukocyte-endothelial adhesion. Activated protein C is
indicated in adult patients with severe sepsis with increased risk of death (APACHE score \(\geq 25\)). In the ENHANCE study, a Phase IIIb open-label study of activated protein C in adult patients with severe sepsis, the survival rate was higher when the drug was initiated on day 1 of acute organ dysfunction compared to day 2 (67% versus 59%; \(p = 0.019\)). (Data available from Eli Lilly, www.xigris.com, accessed September 15, 2005). Survival advantage for early treatment also was noted in a retrospective study of its use in 230 adult septic patients (MERCURY study). However, patients who received activated protein C with single-organ dysfunction and recent surgery (<30 days) had an increased risk of death compared to those who did not (risk ratio 1.52, 95%, confidence interval 1.01–2.33). A trial in children was halted early because of an inability to detect a benefit with the use of activated protein C, although serious bleeding events were not greater with its use. Serious bleeding events occur with activated protein C, and researchers have postulated that those effects would be exaggerated in patients with meningococcal disease. Nonetheless, in a retrospective study, neither pediatric nor adult patients with meningococcal disease receiving activated protein C had increased serious bleeding events compared to those who with meningococcal disease did not receive activated protein C nor to those who received activated protein C but did not have meningococcal disease.24

References
Innate immunity in critical care