CHAPTER 19

Humoral Mediators in Sepsis

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OBJECTIVES

This chapter will:

1. Identify the complex imbalance of proinflammatory and antiinflammatory mediators in sepsis.
2. Describe the biologic actions of the most prominent classes of mediators associated with sepsis.
3. Review the involvement of inflammatory cells (endothelium, monocytes/macrophages, polymorphonuclear neutrophils).

Local infection may develop into a systemic inflammatory response syndrome that encompasses a complex mosaic of interconnected events, including the so-called compensatory antiinflammatory response syndrome.[1] A 2002 hypothesis holds that a defective host innate response may render bacteria resistant to host recognition and defense mechanisms, leading to systemic infection and sepsis.[2] In higher organisms, a variety of host defense mechanisms control the resident microflora and, in most cases, effectively prevent invasive microbial disease. Many microbial pathogens avoid host recognition or dampen the subsequent immune activation through sophisticated interactions with host responses, but some pathogens even benefit from the inflammatory reaction. The defective response of the host may depend on a unique genetic makeup of a pathogen that can render it more resistant to antibiotics or on disturbances in the integrated response of the innate arm and the adaptive arm of the immune system. Differences in reactivity of dendritic cells to microbial molecules through Toll-like receptors (TLRs) are associated with susceptibility and resistance to microbes.[3]

HUMORAL MEDIATORS IN THE PATHOGENESIS OF SEPSIS

A wide range of bacterial-derived molecules have been shown to have a role as humoral mediators in sepsis

pathogenesis: all together, these substances have been classified as pathogen-associated molecular pattern molecules (PAMPs) that include bacterial lipopolysaccharide (LPS), microbial lipopeptides, microbial DNA, porins, formylmethionine (f-Met) proteins, peptidoglycan, and lipoteichoic acid. PAMPs are able to trigger the interaction between the Toll-like receptors and the related molecules (MD-2, MyD88), the principal sensors of the innate immune response.[4,5] In the last decades, several other bacterial-derived products have been shown to play a role in the pathogenic mechanisms of systemic inflammation and sepsis-associated tissue injury. In this setting, quorum sensing (QS) represents a new and heterogeneous class of signal molecules of bacterial colonies regulating microbic growth, biofilm formation, virulence, and antibiotic resistance. Gupta et al. demonstrated that QS from *Pseudomonas aeruginosa* is able to elicit cytokine release.[6] Moreover, QS may regulate the activation of the coagulation cascade and monocyte response to the inflammatory environment.[7]

Other humoral mediators may be released by injured cells during sepsis. Indeed cell injury promotes the release of the so-called endogenous damage-associated molecular pattern molecules (DAMPs) that include RNA/DNA fragments, S100 proteins, heat shock proteins, and the chromatin protein high-mobility box group 1 (HMBG1). HMBG1 is a TLR4 ligand, and its circulating levels have been shown to correlate with organ dysfunction in critically ill patients. Another study has proven that circulating plasma free DNA is a predictor of mortality.[8]

Stimulus-receptor coupling activates different signal transduction pathways, leading to exacerbated generation of cytokines and phospholipase A_2–dependent, arachidonic acid–derived platelet-activating factor (PAF), leukotrienes, and thromboxanes (Fig. 19.1). At the plasma level, activation of the complement (C3a, C5a, and their desarginated products) and coagulation pathways interacts with the process, because products generated in the fluid phase may in turn trigger and sustain cell activation. Other agents play a role in the pathophysiology of sepsis, such as surface-expressed and soluble adhesion molecules, kinins, thrombin, myocardial depressant substances, endorphin, and heat shock proteins.

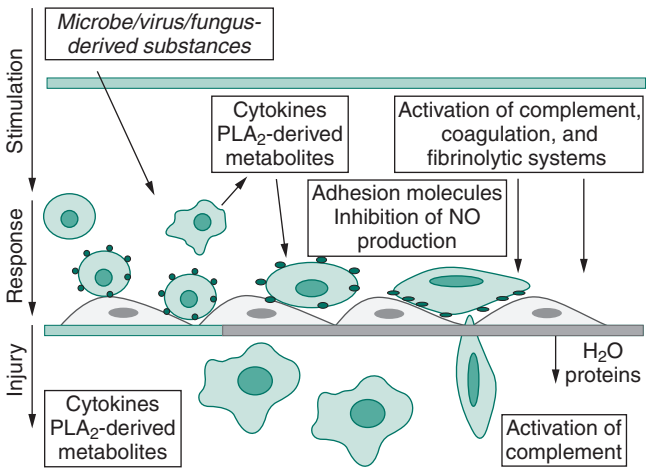

FIGURE 19.1 The drawing schematically represents the leukocyte-endothelium as a result of cytokine production and fluid phase activation. Rolling, tethering, and, finally, attachment of leukocytes to the endothelium layer is mediated by the expression of several adhesion molecules and the platelet-activating factor, release of proteolytic enzymes, and cationic proteins that enhance vascular permeability. Similar mechanisms induce cell activation (mediator production) and complement activation, leading to tissue injury by resident or recruited circulating cells. *H_2O,* Water; *NO,* nitric oxide; *PLA_2,* phospholipase A_2.

In physiologic conditions, the biologic activity of sepsis-associated mediators is under the control of specific inhibitors that may act at different levels. In sepsis, the homeostatic balance is altered and a profound disturbance of relative production of different mediators may be observed (as reviewed by Cavaillon et al.[9]). On one side, the spillover into the circulation of mediators intended to have autocrine or paracrine effects generates systemic effects, including endothelial damage,[10] procoagulant and fibrinolytic effects, complement activities, hemodynamic shock, and vasoparalysis.[11–17] On the other side, monocytes demonstrate a profound inability to produce cytokine when they are challenged with different stimuli ex vivo.[18,19]

In this setting, recent studies showed that different circulating mediators may have a role as predictors of outcome and of disease severity: presepsin, a protein cleaved from the monocyte-specific CD14 receptor complex after binding with LPS is able to discriminate sepsis severity.[20] Angiopoietin-2 (Ang-2) is an endothelial-derived molecule able to increase vascular leakage: serum Ang-2 levels are increased during sepsis and associated with disease severity.[21] Soluble CD40 ligand (sCD40L) shows prothrombotic and proinflammatory properties after binding to its cell surface receptor CD40. Circulating sCD40L levels are significantly higher in septic patients than in controls and in nonsurvivors.[22] Furthermore, several cell fragments are identifiable in plasma of septic patients, including apoptotic bodies, necrotic fragments, and actively released extracellular vesicles (EV). EV are membrane-coated particles carrying tissue specific lipids, proteins mRNA, and microRNA. Sepsis is associated with a massive increase of EV production; the main sources of septic EV are leukocytes, platelets, and injured endothelial cells.[23] EV promote microvascular dysfunction and systemic inflammation.[23] In addition, pathogens can exploit human EV to propagate the infection and may release toxic EV by themselves. Human immunodeficiency virus and herpesviruses can be shuttled by host EV; this allows to spare cell contagion by an immune-privileged shuttle. Hepatitis B virus (HBV) and several bacteria release vesicles as a red herring for host immune system; *Anthrax bacillus* delivers toxins to target cells by its particles.[24] Some parasites, such as *Leishmania*, release immune-inhibiting EV that reduce macrophage activity.[25]

Coagulation Cascade Activation in Sepsis: the Role of Platelet-Activating Factor

Coagulopathy can be seen in essentially all patients with severe sepsis. The earliest signs of consumptive coagulopathy in sepsis are a decrease in protein C level and an increase in D-dimer level. In patients with more severe consumptive coagulopathy, prothrombin time and partial thromboplastin time increase, with drops in fibrinogen level and platelet count. In addition, fibrinolysis also is impaired in severe sepsis.[26] LPSs cause a direct activation of coagulation through the upregulation of tissue factor on the surface of endothelial cells and monocytes. The tissue factor expression in turn activates factor VII of the extrinsic system, leading to thrombin formation and generation of fibrin clots. Thrombin is a multifunctional serine protease with the primary function of cleavage of circulating protein substrates: e.g., conversion of fibrinogen to fibrin monomer or activation of protein C. However, thrombin also has important actions

on cells. It is a potent activator of platelets and causes endothelial cells to deliver the leukocyte adhesion molecule P-selectin to their surfaces, to secrete von Willebrand factor, to elaborate growth factors and cytokines, and to synthesize PAF. Such cellular actions of thrombin may account for its role in controlling early cellular behavior during sepsis.[27]

On the other hand, an indirect activation of the coagulation cascade on the surface of endothelial cells can be triggered by proinflammatory mediators generated by sepsis, including tumor necrosis factor (TNF), interleukin-1 (IL-1), complement fragments, and PAF. In addition, activation of coagulation in sepsis also can occur indirectly throughout the activation of the contact system.

Many experimental and clinical observations suggest that PAF or PAF-like lipids are involved in the unregulated inflammation and pathologic thrombosis observed in septic shock.[28] PAF contributes to acute sequestration and endothelial adhesion of neutrophils and to induction of nitric oxide synthase in experimental endotoxemia.[29] In mice, the overexpression of the PAF receptor increases lethality in response to administration of LPS.[30] However, disruption of the PAF receptor gene in mice caused a marked reduction in systemic anaphylactic symptoms, but mice remained sensitive to bacterial endotoxin.[31]

In humans, several studies have shown the presence of intravascular PAF activity in septic patients.[32–34] PAF is present at high concentrations in blood and bronchoalveolar lavage fluid and occupies specific platelet receptors, and the rate of its catabolism is reduced.[33] In patients with acute renal failure (ARF) associated with septic shock, PAF was present in high concentration in plasma, in association with platelets, and in urine. Plasma concentration of PAF correlated with the severity of renal failure and with indexes of renal inflammatory injury, such as urine IL-6 and IL-8 levels. Interestingly, a negative correlation between concentration of PAF in blood and number of circulating platelets was observed, suggesting a PAF-dependent activation of platelets during septic shock.[32,34]

Proinflammatory and Antiinflammatory Cytokine Network During Sepsis

The pathogenesis of sepsis was described initially as an overproduction of proinflammatory factors in the host. The concept was established on the basis of several studies. The injection of LPS into experimental animals and healthy human subjects reproduces the initial phase of bacterial infection.[35] In human subjects, LPS alters capillary integrity and affects the cardiovascular system,[36] causes production of cytokines,[36–38] and activates the coagulation-fibrinolytic pathways.[39] Peak concentrations of IL-1, TNF-α, IL-6, and IL-8 occur within 2 to 3 hours of LPS infusion.[26,27] Studies on knockout mice have shown that intercellular adhesion molecule-1 (ICAM-1) mutant mice are resistant to the lethal outcome of endotoxin-induced pneumonitis.[40]

What is the relevance of circulating cytokines? The presence or absence of detectable levels of cytokines within biologic fluids reflects a rather complex balance between enhancing and inhibitory signals acting on producer cells, between production and catabolism, and between the binding of cytokines to the target cells and the modulation of their receptors on the cell surface.[41] Furthermore, the presence of cytokines does not necessarily parallel their activity, and a possible interplay between a given cytokine and its relative inhibitor (if known) should be considered. Cavaillon et al.[9] called the expression of circulating cytokines "the tip of the iceberg," implying that neither the presence nor the absence of cytokines can reflect the complex interplay at the tissue level. Despite the fact that the peak concentrations of cytokines may reflect an exacerbated production, these levels do not necessarily represent enhanced bioactivity.

Based on the previous experimental and clinical observations, the concept of sepsis as a simply proinflammatory event has been challenged in recent years (Fig. 19.2).[42] Indeed, after a first phase characterized by a systemic inflammatory response syndrome, cell-associated cytokines in peripheral blood mononuclear cells progressively decrease, and the most relevant decline is observed for TNF-α and IL-1b, IL-6, IL-10, and IL-12.[43–45] Terms such as *monocyte deactivation, immunoparalysis,* and, more simply, *cell hyporesponsiveness* indicate the inability of cells to respond ex vivo to LPS stimuli owing to overproduction of antiinflammatory cytokines. Hyporesponsiveness not only is present in mononuclear cells but also occurs in whole blood,[36] and it is associated with increased plasma levels of IL-10 and prostaglandin E_2, which are potent inhibitors of the production of proinflammatory cytokines.[46] Adib-Conquy et al.[47] demonstrated that, upon LPS activation, peripheral mononuclear cells from patients with systemic inflammatory response syndrome show patterns of nuclear factor-κB (NF-κB) expression that resemble those reported during LPS tolerance: global downregulation of NF-κB in survivors of sepsis and patients with trauma and the presence of large

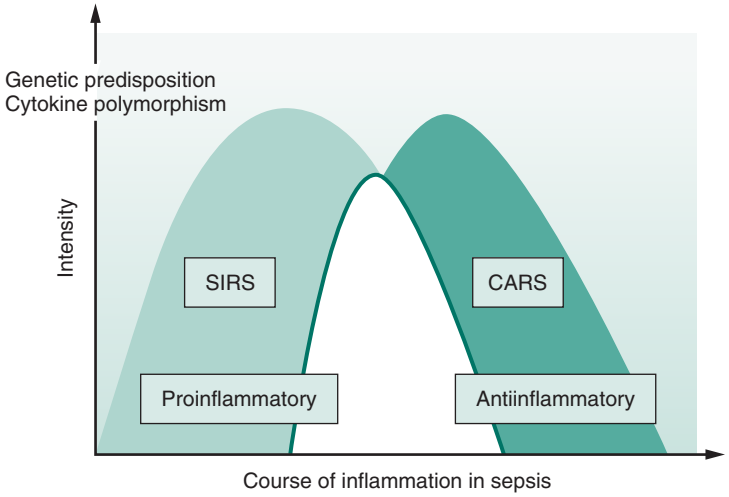

FIGURE 19.2 The first response to an inflammatory response is characterized by the prompt production of several proinflammatory mediators. The extent of this response is important because a reduced early inflammatory response is associated with the unconstrained invasion of the invading organism. The response, which acts at first at a local level, may extend to the systemic level, giving rise to the so-called systemic inflammatory response syndrome (SIRS). As a result of a counterbalanced effect, production of antiinflammatory cytokines begins, which antagonizes the inflammatory response (compensatory antiinflammatory response syndrome [CARS]).

amounts of the inactive homodimer in the nonsurvivors of sepsis. This immune anergy is associated clinically with the late-onset mortality of septic patients.[48]

In intensive care medicine, blocking one mediator has not led to measurable outcome improvement in patients with sepsis.[49] Possibly more rigidly defined subgroups would profit from TNF-antagonizing treatments.[50] On the other hand, it has been shown that antagonizing a cytokine may lead to deleterious consequences, which in turn leads to substantially higher mortality.[51] A low-level TNF-α response seems to be necessary for the host defense to infection,[52] and high levels of TNF-α apparently must be modulated by antiinflammatory feedback. In sepsis, however, impaired regulation may cause an excessive antiinflammatory response, which generates monocyte "immunoparalysis" and exposes the host to further infections. Both processes (inflammation and antiinflammation) are designed to act in response to specific stimuli in a well-balanced fashion defined as immune homeostasis.

Furthermore, the time of therapeutic intervention in sepsis seems to be crucial.[53] Because the network acts as a cascade, early intervention would seem most beneficial. Sepsis shows complex and multiple rises in mediator levels that change over time. Neither single mediator–directed nor one-time interventions therefore seem appropriate. One of the major criticisms of continuous blood purification treatment in sepsis, its lack of specificity, could turn out to be a major strength. Unspecific removal of soluble mediators—be they proinflammatory or antiinflammatory—without complete elimination of their effect may be the most logical and adequate approach to a complex and long-running process such as sepsis to restore immunohomeostasis.[54,55]

The contribution of inflammatory cytokines to the determination of hemodynamic alterations with consequent tissue hypoperfusion and injury acquires a particular relevance in the clinical setting of multiple-organ failure. It has been proven that damaged organs can release a plethora of immune mediators that further contribute to Systemic Inflammatory Response Syndrome (SIRS). In particular, activated kidney tubular cells produce TNF-α, MCP-1, IL-8, IL-6, IL-1β, and TGF-β, regulated on activation, normal T cell expressed and secreted (RANTES), and epithelial neutrophil activating peptide 78 (ENA-78)[56,57]; cardiomyocytes release TNF, interferon-γ and high quantities of natriuretic peptides,[58] injured pneumocytes release IL-1β, IL-6, IL-8 and TNF-α.[59] Moreover, in the context of SIRS, these cells are able to act as immune antigen-presenting cells by expressing MHC II and costimulation molecules, thus worsening tissue inflammation and damage.[60]

The importance of acute kidney injury (AKI) in the induction of functional alterations in distant organs (organ cross-talk) has only lately emerged and is discussed in other chapters. Cytokine production during acute inflammatory disorders such as sepsis is usually the result of the interplay between genetic and environmental factors. Studies on genetic polymorphism of the host immune response may help in identifying patients with a higher susceptibility to developing acute inflammatory disorders.[61] In this clinical setting, a wide range of studies over the last decades tried to identify the specific mediators responsible for organ damage, without success. On the basis of the proven absence of a "magic bullet" to interfere with these detrimental processes, extracorporeal techniques, which achieve unspecific increases in cytokine clearance, have acquired a primary importance.[62–64]

Proinflammatory cytokines induce a direct injury to several cell types, in particular via the activation of the apoptotic processes. It has been shown that lymphocyte apoptosis is a potential mechanism for immunosuppression during sepsis.[65] Endotoxin and inflammatory cytokines induce apoptosis in the myocardium, contributing to cardiac dysfunction.[66]

Several studies demonstrated that apoptosis and necrosis are typical features of acute septic, ischemic, and nephrotoxic ARF.[67] Indeed, ischemia, growth factor deficiency, loss of cell-matrix or cell-cell interactions, oxidant stress, and several pharmacologic compounds (e.g., cisplatin, antibiotics, calcineurin inhibitors) are potential causes of apoptosis in tubular cells. Some other molecules, such as TNF-α, Fas ligand, and angiotensin, can induce tubular apoptosis via the activation of specific receptors located on tubular cells. Given the described increase in inflammatory cytokine levels in AKI, the direct involvement of such cytokines in tissue injury seems probable. Thus inflammatory cytokines are able to activate tubular apoptosis through the upregulation of Fas and the activation of caspases.

Sepsis also is associated to a further pathway of programmed cell death defined as necroptosis. This is a highly immunogenic form of necrosis activated by receptor-interacting protein kinase 1 and 3 (RIPK1, 3) and represents a second-line defense mechanism of the host involving caspase-8 activation. During sepsis, the combination of several humoral signals (such as IFN-γ and TNF-α) can reverse the RIPK/caspase-8 imbalance, promoting necroptosis and, consequently, tissue inflammation.[68]

In addition, these substances cause shedding of tubular cells from the basal membrane, with consequent lumen obstruction and possible back-leakage of tubular fluid in the interstitial spaces.[60]

The altered function induced by different causes in distant organs can result in the systemic release of inflammatory mediators that are potentially involved in kidney injury through the activation of apoptotic processes. Moreover, tubular cells regulate cytokine handling and can cause a further increase in these substances, as reported after renal ischemia-reperfusion injury.[69]

Inflammatory cytokines are also able to effect a variety of nonlethal alterations in epithelial and endothelial cells, in particular loss of cellular polarity and tight junction dysfunction.[70,71] Epithelial tight junction proteins have some major functions, working as a regulatory barrier that separates and maintains biologic fluid compartments of different composition. In addition, tight junction proteins play a key role in the maintenance of polarity, cell growth, and differentiation. In different epithelial districts, inflammatory cytokines are known to increase permeability, via a nitric oxide–dependent mechanism, by altering the expression of some tight junction proteins, such as zonula occludens-1 (ZO-1), ZO-2, ZO-3, and occludin.[72,73] AKI has been shown to involve alterations in tight junction proteins and disruption of actin cytoskeletal fibers. These changes lead to a misleading expression of apicobasal molecules, such as the integrins that anchor tubular cells to basal membrane or Na^+, K^+–ATPase, which regulates tubular sodium handling. Such alterations can contribute to some pathologic manifestations of AKI, including impairment of sodium readsorption and shedding of tubular cells in the lumen, causing obstruction and back-leakage of tubular fluid and activation of the tubule-glomerular feedback.[74] Moreover, these functional alterations of tubular epithelial cells induced by systemic inflammation also may lead to cell dedifferentiation in the so-called epithelial-to-mesenchymal transition, a biologic process at the basis of a maladaptive repair of the kidney finally leading to chronic kidney disease.[60]

PAMPs and DAMPs can be freely filtered by glomeruli reaching tubular cells through the lumen or they alternatively

bind to the basolateral compartment of tubular cells through peritubular capillaries. DAMPs and PAMPs are responsible for the above-described biologic mechanisms of tubular cell injury typical of sepsis-associated AKI, in particular the bioenergetics derangement of cells with loss of mitochondrial function and death.[75] Last, as recently suggested by the XIV acute dialysis quality initiative (ADQI) workgroup in Bogota, Colombia, in the near future the interest may move from humoral mediators to analysis of different types of circulating cells in sepsis[76]: the decrease of HLA-DR expression on monocyte surface is considered a strong predictor of mortality in septic patients.[77] Neutrophil CD64 has been shown to be a highly sensitive and specific marker for systemic infection, sepsis, and tissue injury.[78] In NK cells, NKG2D represent a marker of cell activation, and CD107 expression is used to identify degranulation.[79,80]

Key Points

1. Cytokine production during acute inflammatory disorders such as sepsis is usually the result of the interplay between genetic and environmental factors.
2. Inflammatory cytokines induce a direct injury to several cell types, in particular via the activation of apoptotic processes and leading to simultaneous tissue injury and immunoparalysis.
3. The contribution of inflammatory cytokines to the determination of hemodynamic alterations, with consequent tissue hypoperfusion and injury, acquires a particular relevance in the clinical setting of multiple-organ failure.
4. The "inflammatory milieu" can modulate innate and adaptive immunity through the activation of different cell types.

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