

Sepsis Mediators

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During sepsis, the plasma levels of numerous inflammatory markers are enhanced. Some of these markers are the mediators responsible for the syndromes observed during sepsis as well as for organ dysfunction and eventually death. Their role has been demonstrated in experimental models that employed either transgenic and gene-targeted animals or the use of neutralizing agents. Accordingly, anaphylatoxins generated after complement system activation, factors of coagulation and fibrinolysis, proinflammatory cytokines, chemokines, proteases, lipid mediators, nitric oxide, and cell markers of stress (eg, high mobility group box-1) have been shown to contribute to the deleterious events observed during sepsis. On the other hand, the counter-regulation of the inflammatory process, which involves mediators such as anti-inflammatory cytokines and some neuromediators, can jeopardize the immune status of the host and render the patients more sensitive to nosocomial infections.

Introduction

Infection is associated with a systemic inflammatory response. The severity of the clinical status, ranging from sepsis to severe sepsis to septic shock, depends on the virulence of the pathogen, the use of appropriate antibi-therapy, underlying diseases, and genetic polymorphisms. The frequency and mortality of sepsis in intensive care units are still high. During sepsis, enhanced levels of numerous markers can be detected in the bloodstream as a testimony of the systemic inflammatory response. Usually, the highest levels of these markers correlate with the poorest outcome; however, this correlation does not prove that they are the active mediators responsible for sepsis syndromes. This review focuses on mediators rather than markers. Experiments with neutralizing agents (ie, antibodies, antagonists, drugs) or the use of transgenic or gene-targeted mice (either overexpressing the molecule or knockout [KO] for

the gene of a given mediator or its receptor) have helped to define the involvement of each individual molecule in the occurrence of sepsis syndromes.

Complement System

The complement system plays a pivotal role during sepsis. The major purpose of the activation of the complement system through its three pathways is to remove and/or destroy pathogens. However, some of the derived compounds released after activation of complement favor the inflammatory process, as illustrated by their ability to increase vascular permeability and contribute to granulocyte and monocyte recruitment within inflamed tissues.

Anaphylatoxins C3a and C5a are involved in infection control and inflammatory regulation. Both pro- and anti-inflammatory properties have been attributed to C3a [1]. Anaphylatoxin C5a favors the synthesis and release of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 β , IL-6, and IL-8 from human leukocytes [2], and it can enhance phagocytosis, induce oxidative burst, and favor the release of granular enzymes from neutrophils. In the lung, C5a enhances inflammatory response, particularly by its action on alveolar epithelial cells. C5a has also been found to be a vasodilator, to enhance the expression of adhesion molecules, and to activate the coagulation system. Furthermore, C5a has a major role in cardiac dysfunction during sepsis, participating in septic cardiomyopathy [3•]. Excessive production of C5a may compromise the host defenses, and high levels of plasma C5a in sepsis patients correlate with poor outcome [4••]. In some models of shock, C5-deficient animals are more resistant to the effects of TNF and lipopolysaccharide (LPS) infusions than controls. Studies of sepsis in animal models have shown that blockade of C5a attenuates physiologic perturbations and prevents the development of acute respiratory distress syndrome and multiple organ failure. After cecal ligation and puncture (CLP), a model of peritonitis and sepsis, an increased expression of C5a receptor occurs in most tissues including lungs, liver, kidneys, and heart. Most importantly, blockade of C5a receptor is highly protective [4••].

C4 or C3 KO mice are more susceptible to endotoxin, with an increased consumption of C1q inhibitor. C1q inhibitor is a plasma glycoprotein, which participates in both complement regulation and contact system activation. Interestingly, supplementation with C1q inhibitor prevents

endotoxin-induced increase of vascular permeability [5] and protects against LPS-induced mortality. In addition, C1q inhibitor protects mice by interacting directly with LPS [6]. Thus, despite the fact that circulating inactivated form of C1q inhibitor is increased in sepsis and is associated with a pejorative evolution of sepsis, animal models have suggested a protective role of this molecule.

Coagulation and Fibrinolysis

Disorders of coagulation are common in sepsis, and 30% to 50% of patients with the most severe clinical form have disseminated intravascular coagulation. Tissue factor is the link between inflammation and coagulation. It interacts with factor VII and increases the production of fibrin through activation of prothrombin in thrombin. In healthy volunteers challenged with LPS and patients with sepsis, tissue factor expression is enhanced, and its increased membrane expression on monocytes appears as a prognostic factor of poor outcome in sepsis [7]. Factor VII strongly colocalizes with tissue factor, which correlates with fibrin deposition, mainly at the vascular bifurcations. Thrombin signaling in endothelial cells results in changes in cell shape, cell permeability, proliferative response, and leukocyte adhesion. Thrombin is inactivated by antithrombin, but during sepsis, levels of antithrombin are reduced. Numerous animal models of sepsis have revealed beneficial effects of antithrombin. Factor XIII is involved in fibrin stabilization. Whereas factor XIII subunit A and its cross-linking are decreased in septic patients, the specific activity of factor XIII is increased in these patients [8]. Neither subunit A nor cross-linking activity is associated with patient severity; nevertheless, specific activity of factor XIII is strongly associated with severity and fatality in the septic group [8].

The study by Bernard et al. [9] opened a new area of investigation when it showed that activated protein C (APC) displays beneficial effects in human septic shock. However, controversies currently exist regarding the interest of this molecule. Protein C is synthesized by the liver and circulates as an inactive zymogen. During sepsis, protein C synthesis is reduced, and endothelial shedding is associated with a decrease in thrombomodulin expression. These phenomena lead to a significant decrease in APC, which eventually results in a prothrombotic state. Decreased levels of protein C during sepsis are associated with a poor outcome in patients [10]. It has been postulated that protein C plays a central role in linking inflammatory and coagulation processes. APC seems to possess anti-inflammatory properties and counteract fibrinolysis inhibition.

Although coagulation is increased during sepsis, the mechanisms that favor fibrinolysis are reduced. Thrombin-activatable fibrinolysis inhibitor (TAFI) is an inhibitor of the fibrinolytic system. TAFI is able to inhibit C3a and C5a. In mice, injection of LPS or *Escherichia coli* induces

an enhanced plasma TAFI activity. On the other hand, TAFI deficiency modifies neither coagulation markers during sepsis nor fibrin deposition in liver and lung tissue. However, TAFI-deficient mice are protected from liver necrosis during peritonitis. In humans, TAFI was shown to be decreased in plasma from healthy subjects injected with LPS [11]. Plasminogen activator inhibitor-1 is another inhibitor of fibrinolysis, which acts by inhibiting the transformation of plasminogen into active plasmin. Sepsis is associated with increased levels of plasminogen activator inhibitor-1, which seem to be a pejorative factor in patients with sepsis complicated by intravascular coagulation [12] but a good prognosis marker during pneumonia [13].

Cytokines, Chemokines, and Growth Factors

Among effector molecules, cytokines play a central role in the initiation of the inflammatory response. Cytokines and chemokines are produced in response to pathogens and endogenous alarm signals ("alarmins"). They allow communication, coordination, and activation of immune cells that are a prerequisite to fight infection. However, they also contribute to the pathogenesis of endotoxemia or septic shock. In septic patients, cytokines are produced in excess and are therefore detectable in the blood, where they are normally absent [14]. Yet, circulating cytokines are only the tip of the iceberg [15].

IL-1 β and TNF play a synergistic role in orchestrating the inflammatory response. TNF and IL-1 enhance procoagulant activity of vascular endothelial cells, activate neutrophils, and increase gene expression for adhesion molecules, which in turn can worsen tissue injury during sepsis. TNF has been shown to contribute to mortality in animal models with either gram-positive or gram-negative bacteria [16], and numerous studies in animal models have established that neutralization of TNF was beneficial before injection of LPS or bacteria. However, it is worth mentioning that the major role of TNF in LPS-induced mortality in TNF-KO mice was only achieved when LPS was injected together with the hepatotoxic agent galactosamine [17].

Injection of LPS administered to IL-1 β -deficient mice produced similar observations as in wild-type mice, probably because other IL-1-like cytokines (ie, IL-1 α , IL-18, IL-33) can compensate for the absence of IL-1 β . Indeed, in contrast, in the absence of caspase-1, the maturation enzyme required to get biologically active IL-1 β , IL-18, and IL-33, mice are resistant to endotoxic shock. Sepsis or LPS injection in healthy volunteers led to detectable levels of circulating TNF and IL-1 in plasma. TNF and IL-1 levels are poorly associated with outcome in septic patients, although the long-lasting presence of detectable circulating TNF levels correlates with mortality. In meningococcal sepsis, plasmatic levels of TNF correlate with pejorative prognosis [18].

IL-12 is a heterodimeric cytokine made of p40 and p35 chains. IL-12 shares with IL-18 the capacity to

induce the production of interferon (IFN)- γ . IL-12 is known to play a major role in defense against bacterial infection, and IL-12 deficiency decreases resistance to polymicrobial sepsis due to CLP [19]. Neutralization of IL-18 protected mice against lethal *E. coli* or *Salmonella typhimurium* endotoxemia [20], and IL-18-deficient mice showed decreased sensitivity toward LPS-induced shock [21]. IFN- γ possesses well-known proinflammatory and antibacterial properties. Particularly, IFN- γ enhances phagocytosis and free radical production and increases bactericidal activity of macrophages and neutrophils. However, blockade of IFN- γ is associated with a better prognosis in animal models of sepsis and is associated with a decreased bacterial load and lower systemic inflammation [22]. This phenomenon was partially attributed to an increase in fibrin deposition that could be a protective factor against pathogen dissemination [22]. Echtenacher et al. [23] showed that concomitant injection of IFN- γ at the time of CLP increases lethality. Indeed, simultaneous injection of a nonlethal dose of IFN- γ together with a nonlethal dose of LPS or TNF induces lethality. Of note, IFN- γ is known to be an effective inducer of high mobility group box-1 (HMGB-1) protein production and release by monocytes/macrophages in vitro (see later discussion). In a CLP model of sepsis, inhibition of IFN- γ is associated with decreased HMGB-1 expression in the peritoneum and increased survival of animals [24].

IL-23 shares with IL-12 its p40 subunit. IL-23 is involved in chronic inflammatory response to infection that involves adaptive immune cells. Interestingly, IL-23 seems to be produced early in response to *Pseudomonas aeruginosa*, and its blockade by antibodies is associated with dose-dependent improvement in survival [25]. Similarly to IL-23, IL-27 is a heterodimeric cytokine made of an IL-12 p40-related Epstein-Barr virus-induced gene 3 (EBI3) and a p28 chain. IL-27 mRNA is expressed after CLP in lungs and spleen. EBI3-deficient mice are resistant to CLP-induced lethality, and neutralization of IL-27 protects from sepsis [26•].

Macrophage migration inhibitory factor (MIF) is produced in response to TNF and IFN- γ and more surprisingly also in response to glucocorticoids. MIF is preformed within leukocytes and can also be produced by the pituitary gland. MIF directly or indirectly promotes the production or expression of a large panel of proinflammatory cytokines. MIF-deficient mice display a significant improvement in survival after a challenge with either LPS or *Staphylococcus aureus* enterotoxin B. During peritonitis, MIF increases rapidly in the peritoneum, and its inhibition protects mice from death [27]. In a two-hit model, with pancreatitis followed by an injection of endotoxin, MIF is associated with a pejorative evolution of acute lung injury [28•]. Increased levels of MIF have been detected in the blood of patients with severe sepsis or septic shock and correlate with severity [27].

Chemokines control leukocyte trafficking during homeostasis and inflammation. Clinical studies identified elevated levels of both CXC (eg, CXCL8 [IL-8]), and CC chemokines such as CCL2 associated with human sepsis and acute lung injury. Elevated plasma levels of all chemokines but RANTES (regulated on activation, normal T-cell expressed and secreted; CCL5) correlate with poor outcome [14]. In a sepsis model, macrophage inflammatory protein-2 (CXCL2) increases severity, whereas blocking its activity decreases mortality. Similarly, CXCL-receptor inhibitor improves survival in sepsis [29•] and reduces HMGB-1-induced lung inflammation and injury. In a sepsis model of peritonitis, the neutralization of monocyte chemoattractant protein (MCP)-1 (CCL2) decreased the production of IL-13 and IL-12 and increased the production of TNF and IL-10. In an LPS-induced lethality model, administration of MCP-1 protected mice, whereas the neutralization of MCP-1 with antibodies increased mortality. Indeed, the relative contribution of chemokines to the pathophysiologic events associated with sepsis is linked to their capacity to recruit inflammatory cells within tissues. In contrast, their presence within the bloodstream may limit the inflammatory process by unpriming circulating cells to further chemoattractant signals.

So far, very little has been demonstrated concerning the role of growth factor in sepsis, except for the vascular endothelial growth factor (VEGF). Yano et al. [30•] showed increased levels of VEGF in patients with sepsis. They also demonstrated that an overexpression of soluble Flt-1 (VEGF receptor) limited inflammatory markers in a mouse model of endotoxemia and increased survival after CLP or LPS injection.

Proteases

During sepsis, proteases are released by activated leukocytes and play an important role in inflammatory host response. Their role seems dependent on the pathogen. For example, mice deficient for elastase are more susceptible to gram-negative peritoneal infections, whereas no difference in mortality was shown during peritoneal gram-positive infection. On the other hand, elastase is associated with organ dysfunction, because its inhibition decreases this phenomenon [31].

Among proteases, matrix metalloproteinases (MMPs) are known to be involved in tissue remodeling (ie, the degradation and remodeling of all components of extracellular matrix) and inflammatory processes. Among them, MMP-9 is of paramount importance during sepsis. Serum levels of MMP-9 have been shown to rapidly increase in an *E. coli*-induced bacteremia in baboons and following injection of endotoxin in healthy humans. These increases are observed not only in serum, but also in lung, liver, and peritoneal fluid in a CLP model of peritonitis [32]. Moreover, MMP-9 levels correlate with severity and mortality of sepsis in humans [33]. Interestingly, whereas MMP-9 deficiency protects mice against mortality in an endotoxin

Table 1. Immune dysregulation during sepsis is characterized by an exacerbated production of proinflammatory mediators that lead to deleterious effects and lethality, as well as an exacerbated production of anti-inflammatory mediators that contribute to an immune-suppressive status

	Mediators contributing to tissue injury, organ or system dysfunction, and eventually death*	Mediators favoring immune suppression
Cytokines	Tumor necrosis factor IL-1, IL-12, IL-15, IL-1 β , IL-22, IL-27 IFN- γ , IFN- β Granulocyte-macrophage colony-stimulating factor Leukemia inhibitory factor Macrophage migration inhibitory factor Some chemokines: CXCL8 (IL-8), CCL5, CXCR1 and 2 ligands, CCR1 ligands, CCR4 ligands [†]	IL-10 IL-13 Transforming growth factor- β
Growth factors	Vascular endothelial growth factor	
Cell markers of stress	High mobility group box-1 protein Crystal uric acid S100	Heat shock proteins
Plasma factors	Ligand of TREM-1 Anaphylatoxin C5a Mannose-binding lectin	Ligand of TREM-2
Lipid mediators	Prostaglandins Leukotrienes Platelet-activating factor Oxidized phospholipids	Prostaglandins
Hormones	-	Glucocorticoids
Neuromediators	Substance P Neurokinins Noradrenalin	Adrenalin, acetylcholine α -melanocyte stimulating hormone Vasoactive intestinal peptide Urocortin, cortistatin, adrenomedullin
Enzymes	Cyclooxygenase-2, 5 ^l -lipoxygenase Phospholipase A ₂ , metalloproteinase-9 Elastase, mast cell dipeptidyl peptidase 1 Glycogen synthase kinase-3 Inducible nitric oxide synthase Nicotinamide adenine dinucleotide phosphate oxidase	
Coagulation factor and fibrinolysis	Tissue factor, thrombin Thrombin activatable fibrinolysis inhibitor	
Free radicals	Nitric oxide, superoxide anion	
Gas	Hydrogen sulfide	
Ion transporter	Na ⁺ K ⁺ Cl ⁻ transporter	
Purine nucleoside	Adenosine (via A2A receptor)	Adenosine (via A2A receptor)

*As demonstrated in animal models with the help of specific antibodies, inhibitors, or antagonists or with knockout mice.

[†]Either CCL17 or CCL22.

IFN—interferon, IL—interleukin, TREM—triggering receptor expressed on myeloid cells.

model, such a deficiency is associated with an enhancement of bacterial growth and bacterial dissemination following CLP [32]. Other enzymes can also contribute to the deleterious effects observed during sepsis (Table 1).

Lipid Mediators

Proinflammatory cytokines induce the synthesis of phospholipase A2 (PLA2), inducible cyclooxygenase-2, 5'-lipoxygenase, and acetyltransferase, which contribute to eicosanoids synthesis. Leukotrienes, 5'-lipoxygenase products, are increased in endotoxemic and septic animals. These factors promote inflammation, alter vasomotor tone, and increase blood flow and vascular permeability. Blocking the synthesis of leukotrienes or using 5'-lipoxygenase-deficient mice allowed researchers to demonstrate the deleterious effect of this eicosanoid in a mouse sepsis model [34]. Mice deficient in PLA2 are resistant to endotoxin, and in vivo inhibition of PLA2 decreases neutrophil infiltration of the lungs and deterioration of gas exchanges during endotoxin or zymosan challenge in mice. Cyclooxygenase-2-deficient mice are also resistant to endotoxin-induced inflammation and death [35].

Platelet-activating factor (PAF) is released by many cell types, such as platelets, endothelial cells, macrophages, and neutrophils. Blood levels of PAF are elevated during septic shock, whereas the activity of PAF-acetylhydrolase, its inhibitor, decreases in humans during experimental endotoxemia or sepsis [36]. Inhibition of PAF before endotoxin challenge in healthy humans is associated with a decreased intensity of symptoms and decreased proinflammatory cytokine levels. Conversely, transgenic mice that overexpress PAF are more susceptible to LPS challenge, but surprisingly, mice lacking PAF receptor display a similar susceptibility to endotoxin as their wild-type counterparts. In human sepsis, targeting PAF has failed to demonstrate any beneficial effects.

Nitric Oxide

Nitric oxide (NO) involved in sepsis is produced by inducible NO synthase (iNOS), an enzyme produced in response to endotoxin or inflammatory cytokines. Enhanced iNOS activity in inflamed tissues and vessel walls of sepsis patients has been demonstrated [37]. NO production has been involved in many pathophysiologic processes during sepsis or endotoxemia models, and NO is rendered responsible for multiorgan failure [38]. On the other hand, NO was shown to reduce neutrophil migration to infected tissues by inhibiting leukocyte rolling. iNOS-deficient mice showed a very high mortality rate after *S. aureus* infection or during polymicrobial sepsis [39]. iNOS deficiency is sometimes associated with improvement in metabolic perturbations, such as acidosis and hypotension [40]. In contrast, iNOS-deficient mice and the wild-type group have similar sensitivities to LPS. NO and coagulation are

interrelated during inflammation and sepsis. Inhibition of iNOS was associated with a decrease in tissular plasminogen activator (tPA) drop. One of the major deleterious effects of NO is probably its ability to alter epithelial tight junction, responsible for the pulmonary, liver, and gut dysfunction in endotoxemic mice [41•].

Cellular Markers of Stress and Soluble Cell Surface Markers

HMGB-1 is a nuclear protein present in almost all eukaryotic cells, and it acts as a transcription factor-like protein regulating the expression of several genes. HMGB-1 is a late mediator in endotoxemia and sepsis [42]. Levels of HMGB-1 are correlated with severity during pneumonia in humans [43] and other sepsis. Passive immunization with neutralizing anti-HMGB-1 antibodies prevents lethality from sepsis, even when administered 24 hours after the induction of peritonitis [42]. HMGB-1 mediates hepatic injury after murine liver ischemia reperfusion in a Toll-like receptor 4-dependent fashion [44]. Importantly, HMGB-1 appears to be the link between the occurrence of apoptosis, organ damage, and lethality in sepsis [45••].

Triggering receptor expression on myeloid cells (TREM)-1 is expressed on the surface of monocytes and neutrophils. Its expression can be upregulated by LPS. Its ligand remains to be identified, but its activation leads to cell activation in synergy with different microbial-derived products [46]. This protein may be released from the cell surface, and large amounts of soluble TREM-1 are found in the body fluids of sepsis patients. Soluble TREM-1 has been suggested to be a marker of infection, although recent studies have questioned that specificity [47].

CD163 is a hemoglobin scavenger receptor, which exists exclusively on monocytes and macrophages and which is shed after stimulation with LPS. Accordingly, the soluble form of CD163 is increased in plasma during sepsis. It plays a role in the anti-inflammatory response [48], as do heat shock proteins released by stressed cells.

Lipoproteins and Lipopolysaccharide Inhibitors

Besides their function in lipid transport and metabolism, lipoproteins play a role during sepsis. LPS interacts with lipoproteins and is neutralized and shuttled to the liver. Apolipoprotein AE-deficient mice are more susceptible to LPS or *Klebsiella pneumoniae* challenge. As well, apolipoprotein AI can protect mice against mortality [49].

CD14 exists as a membrane form and is part of the LPS receptor. It also exists as a soluble form known to favor the responsiveness to LPS of cells that lack membrane CD14. However, the role of soluble CD14 remains controversial. In a transgenic mouse model expressing different copy numbers of the human CD14 gene, it was shown that animals expressing the highest levels of soluble CD14 were less sensitive to LPS-induced lethality [50].

In human sepsis, increased levels of circulating soluble CD14 correlates with mortality.

LPS-binding protein (LBP) is an acute phase protein, levels of which are enhanced during sepsis. Despite its ability to shuttle the LPS to CD14, LBP seems to protect mice from septic shock during gram-negative bacterial challenge, whereas LBP-deficient mice are more susceptible to *Salmonella* challenge than are wild-type mice. Bactericidal/permeability-increasing protein shares more than 40% homology with LBP. It attenuates LPS-mediated endothelial damage and IL-6 production, as well as LPS-mediated NO, IL-1, IL-6, IL-8, and TNF production by macrophages or in whole blood.

Lactoferrin, lysozyme, hemoglobin, surfactant proteins A and D, and antimicrobial cationic peptide (CAP18 and CAP37) also bind endotoxins and modify their capacity to initiate an inflammatory process.

Anti-inflammatory Mediators

Although sepsis is associated with a large production of proinflammatory cytokines within the area of infection, the systemic response could be principally an anti-inflammatory one [51]. Indeed, large amounts of anti-inflammatory cytokines can be found in the plasma of sepsis patients, with the highest levels often associated with the poorest outcome. They downregulate the production of proinflammatory cytokines, and they favor the release of soluble TNF receptors and the production of IL-1 receptor antagonist (IL-1Ra). These effects are evident for IL-10, IL-4, IL-13, transforming growth factor- β , and IFN- α . All have been shown to protect animals from sepsis or endotoxin-induced shock. However, they probably also contribute to the altered immune status observed in septic patients. High levels of soluble TNF receptor, soluble IL-1 receptor, and IL-1Ra are found in plasma of sepsis patients. IL-1Ra-deficient mice are highly susceptible to endotoxin-induced death, and conversely, mice that overexpress IL-1Ra are relatively protected. Unfortunately, human trials have failed to find any beneficial effects for IL-1Ra. IL-6 is probably one of the best markers of severity. It induces the release of cortisol, IL-10, IL-1Ra, and acute-phase proteins. Accordingly, it can be considered an anti-inflammatory cytokine. However, IL-6 also possesses some proinflammatory properties, as illustrated by its role in myocardial depression [52].

Heme oxygenase (HO)-1 is induced by IL-10 and plays a protective role against oxidative stress. Compared to wild-type mice, HO-1-deficient mice are more sensitive to endotoxin. HO-1 has been shown to exert beneficial effects in ischemia/reperfusion and hemorrhagic shock models.

Angiopoietins are a class of angiogenic growth factors that act selectively on endothelial cells. Angiopoietin (Ang)-1 possesses anti-inflammatory properties reducing leukocyte-endothelial cell adhesion and transmigration, and it protects mice from endotoxin-induced mortality

[53]. Ang-2 is another angiopoietin, which recognizes the same receptor as Ang-1 but antagonizes this latter molecule by blocking signal transduction. Ang-2 levels are also increased during sepsis in humans, and interestingly, these levels correlate with disease severity scores and with levels of inflammatory cytokines, C-reactive protein, and procalcitonin [54].

Angiotensin converting enzyme (ACE)-2 inactivates angiotensin II and is a negative regulator of the renin-angiotensin system. Interestingly, ACE-2 is involved in the limitation of lung injury during a peritonitis model of sepsis, in contrast with ACE-1, which seems to worsen lung disease in the same conditions [55].

Adenosine acts on specific A₂ receptors and inhibits numerous neutrophil functions such as phagocytosis, generation of superoxide anion, arachidonic acid release, leukotriene B₄ biosynthesis, and adhesion to endothelial cells. Adenosine also inhibits IL-12 and TNF production by macrophages, and it seems to play a protective role against tissue damage. Finally, leptin, a homeostatic protein involved in body weight and satiety, has been shown to increase during LPS challenge, probably in response to IL-1 β . Leptin has a protective role in mice injected with LPS or TNF [56].

Neuromediators

Except substance P, neurokinins, and norepinephrine, most neuromediators dampen the inflammatory process. Substance P and neurokinin A derive from the preprotachykinin-A gene. Mortality due to CLP is decreased and the onset of mortality is delayed in preprotachykinin-A-deficient mice compared to wild-type mice [57]. Norepinephrine increases TNF production via α ₂-adrenergic receptors, whereas epinephrine via β ₂-adrenergic receptors decreases production of TNF, IL-6, and NO in response to LPS and potentiates IL-10 production [58].

Adrenomedullin is a neuropeptide structurally related to corticotropin-releasing factor, which increases in response to LPS injection with an enhanced expression in both plasma and viscera. Adrenomedullin inhibits TNF expression [59] and stimulates IL-6 production by macrophages in response to LPS. Increasingly, observations have implicated the vagus nerve in the downregulation of inflammation. Borovikova et al. [60] established the key role played by acetylcholine and showed that the α ₇-nicotinic receptor contributes to the central modulation and integration of vagal regulation during inflammation. On the other hand, peripheral muscarinic receptors do not have such anti-inflammatory properties [61].

Conclusions

Syndromes associated with sepsis are due to the infection itself as well as the host response to the pathogens.

This response is mediated through a complex network of pro- and anti-inflammatory mediators. Although each pro-inflammatory mediator has its own potential deleterious effect, the synergy between them and with the microbial compounds is mainly responsible for the occurrence of organ dysfunction and eventually death. Numerous animal models have allowed the identification of the mediators contributing to the deleterious effects observed during sepsis. However, caution should be exercised, because the nature of the experimental model greatly influences the conclusions. Despite positive results in animal models, the therapeutic targeting of these mediators has been extremely disappointing in human settings. This disappointment does not mean that they are not involved in the syndromes associated with human sepsis. Indeed, because of the tremendous synergy that exists between all these mediators, the strategy of targeting one only molecule appears a bit too simplistic. On the other hand, the regulatory anti-inflammatory mediators may jeopardize the patient to a second infection. The control of sepsis as a way to respect inflammation equilibrium should be the main concept of therapeutic intervention in the future.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Haeffner-Cavaillon N, Cavaillon JM, Laude M, Kazatchkine MD: C3a(C3adesArg) induces production and release of interleukin 1 by cultured human monocytes. *J Immunol* 1987, 139:794–799.
 2. Cavaillon JM, Fitting C, Haeffner-Cavaillon N: Recombinant C5a enhances interleukin 1 and tumor necrosis factor release by lipopolysaccharide-stimulated monocytes and macrophages. *Eur J Immunol* 1990, 20:253–257.
 - 3• Niederbichler AD, Hoesel LM, Westfall MV, et al.: An essential role for complement C5a in the pathogenesis of septic cardiac dysfunction. *J Exp Med* 2006, 203:53–61.
- A major contribution in the understanding of myocardial dysfunction during sepsis.
- 4•• Ward PA: The dark side of C5a in sepsis. *Nature Rev Immunol* 2004, 4:133–142.
- An important review on C5a by the leader in the field who convinced the scientific community that C5a plays a central role in sepsis.
5. Liu D, Zhang D, Scaffidi J, et al.: C1 inhibitor prevents Gram-negative bacterial lipopolysaccharide-induced vascular permeability. *Blood* 2005, 105:2350–2355.
 6. Liu D, Cai S, Gu X, et al.: C1 inhibitor prevents endotoxin shock via a direct interaction with lipopolysaccharide. *J Immunol* 2003, 171:2594–2601.
 7. Osterud B, Flaegstad T: Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. *Thromb Haemost* 1983, 49:5–7.
 8. Zeerleder S, Schroeder V, Lammler B, et al.: Factor XIII in severe sepsis and septic shock. *Thromb Res* 2007, 119:311–318.
 9. Bernard GR, Vincent JL, Laterre PF, et al.: Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001, 344:699–709.
 10. Yan SB, Helterbrand JD, Hartman DL, et al.: Low levels of protein C are associated with poor outcome in severe sepsis. *Chest* 2001, 120:915–922.
 11. Verbon A, Meijers JC, Spek CA, et al.: Effects of IC14, an anti-CD14 antibody, on coagulation and fibrinolysis during low-grade endotoxemia in humans. *J Infect Dis* 2003, 187:55–61.
 12. Madoiwa S, Nunomiya S, Ono T, et al.: Plasminogen activator inhibitor 1 promotes a poor prognosis in sepsis-induced disseminated intravascular coagulation. *Int J Hematol* 2006, 84:398–405.
 13. Renckens R, Roelofs JJ, Bonta PI, et al.: Plasminogen activator inhibitor type 1 is protective during severe Gram-negative pneumonia. *Blood* 2007, 109:1593–1601.
 14. Cavaillon JM, Adib-Conquy M, Fitting C, et al.: Cytokine cascade in sepsis. *Scand J Infect Dis* 2003, 35:535–544.
 15. Cavaillon JM, Munoz C, Fitting C, et al.: Circulating cytokines: the tip of the iceberg? *Circ Shock* 1992, 38:145–152.
 16. Sappington PL, Yang R, Yang H, et al.: HMGB1 increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice. *Gastroenterology* 2002, 123:790–802.
 17. Amiot F, Fitting C, Tracey KJ, et al.: Lipopolysaccharide-induced cytokine cascade and lethality in LT alpha/TNF alpha-deficient mice. *Mol Med* 1997, 3:864–875.
 18. Vermont CL, Hazelzet JA, de Kleijn ED, et al.: CC and CXC chemokine levels in children with meningococcal sepsis accurately predict mortality and disease severity. *Crit Care* 2006, 10:R33.
 19. Moreno SE, Alves-Filho JC, Alfaya TM, et al.: IL-12, but not IL-18, is critical to neutrophil activation and resistance to polymicrobial sepsis induced by cecal ligation and puncture. *J Immunol* 2006, 177:3218–3224.
 20. Netea MG, Fantuzzi G, Kullberg BJ, et al.: Neutralization of IL-18 reduces neutrophil tissue accumulation and protects mice against lethal *Escherichia coli* and *Salmonella typhimurium* endotoxemia. *J Immunol* 2000, 164:2644–2649.
 21. Hochholzer P, Lipford GB, Wagner H, et al.: Role of interleukin-18 (IL-18) during lethal shock: decreased lipopolysaccharide sensitivity but normal superantigen reaction in IL-18-deficient mice. *Infect Immun* 2000, 68:3502–3508.
 22. Qiu G, Gribbin E, Harrison K, et al.: Inhibition of gamma interferon decreases bacterial load in peritonitis by accelerating peritoneal fibrin deposition and tissue repair. *Infect Immun* 2003, 71:2766–2774.
 23. Echtenacher B, Freudenberg MA, Jack RS, Mannel DN: Differences in innate defense mechanisms in endotoxemia and polymicrobial septic peritonitis. *Infect Immun* 2001, 69:7271–7276.
 24. Yin K, Gribbin E, Wang H: Interferon-gamma inhibition attenuates lethality after cecal ligation and puncture in rats: implication of high mobility group box-1. *Shock* 2005, 24:396–401.
 25. Belladonna ML, Vacca C, Volpi C, et al.: IL-23 neutralization protects mice from Gram-negative endotoxemic shock. *Cytokine* 2006, 34:161–169.
 - 26• Wirtz S, Tubbe I, Galle PR, et al.: Protection from lethal septic peritonitis by neutralizing the biological function of interleukin 27. *J Exp Med* 2006, 203:1875–1881.
- An important work that addressed the contribution of newly discovered cytokines in the field of sepsis.
27. Calandra T, Echtenacher B, Roy DL, et al.: Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nat Med* 2000, 6:164–170.
 - 28• Matsuda N, Nishihira J, Takahashi Y, et al.: Role of macrophage migration inhibitory factor in acute lung injury in mice with acute pancreatitis complicated by endotoxemia. *Am J Respir Cell Mol Biol* 2006, 35:198–205.
- Underlies the importance of the two-hit concept in multiorgan failure development.

29. Kaneider NC, Agarwal A, Leger AJ, Kuliopulos A: Reversing systemic inflammatory response syndrome with chemokine receptor peptiduccins. *Nat Med* 2005, 11:661–665.
- An elegant approach to demonstrate the deleterious effects of chemokines during sepsis.
30. Yano K, Liaw PC, Mullington JM, et al.: Vascular endothelial growth factor is an important determinant of sepsis morbidity and mortality. *J Exp Med* 2006, 203:1447–1458.
- Describes for the first time the possibility that a growth factor could contribute to sepsis syndromes.
31. Nakamura A, Mori Y, Hagiwara K, et al.: Increased susceptibility to LPS-induced endotoxin shock in secretory leukoprotease inhibitor (SLPI)-deficient mice. *J Exp Med* 2003, 197:669–674.
32. Renckens R, Roelofs JJ, Florquin S, et al.: Matrix metalloproteinase-9 deficiency impairs host defense against abdominal sepsis. *J Immunol* 2006, 176:3735–3741.
33. Yassen KA, Galley HF, Webster NR: Matrix metalloproteinase-9 concentrations in critically ill patients. *Anaesthesia* 2001, 56:729–732.
34. Benjamim CF, Canetti C, Cunha FQ, et al.: Opposing and hierarchical roles of leukotrienes in local innate immune versus vascular responses in a model of sepsis. *J Immunol* 2005, 174:1616–1620.
35. Ejima K, Layne MD, Carvajal IM, et al.: Cyclooxygenase-2-deficient mice are resistant to endotoxin-induced inflammation and death. *Faseb J* 2003, 17:1325–1327.
36. Gomes RN, Bozza FA, Amancio RT, et al.: Exogenous platelet-activating factor acetylhydrolase reduces mortality in mice with systemic inflammatory response syndrome and sepsis. *Shock* 2006, 26:41–49.
37. Annane D, Sanquer S, Sebille V, et al.: Compartmentalised inducible nitric-oxide synthase activity in septic shock. *Lancet* 2000, 355:1143–1148.
38. Asakura H, Asamura R, Ontachi Y, et al.: Selective inducible nitric oxide synthase inhibition attenuates organ dysfunction and elevated endothelin levels in LPS-induced DIC model rats. *J Thromb Haemost* 2005, 3:1050–1055.
39. Benjamim CF, Silva JS, Fortes ZB, et al.: Inhibition of leukocyte rolling by nitric oxide during sepsis leads to reduced migration of active microbicidal neutrophils. *Infect Immun* 2002, 70:3602–3610.
40. Matejovic M, Krouzecky A, Radej J, et al.: Coagulation and endothelial dysfunction during longterm hyperdynamic porcine bacteremia—effects of selective inducible nitric oxide synthase inhibition. *Thromb Haemost* 2007, 97:304–309.
41. Han X, Fink MP, Uchiyama T, et al.: Increased iNOS activity is essential for pulmonary epithelial tight junction dysfunction in endotoxemic mice. *Am J Physiol Lung Cell Mol Physiol* 2004, 286:L259–267.
- A smart demonstration of the involvement of NO during sepsis, leading to the alteration of epithelial integrity, of paramount importance in the lung and other tissues.
42. Yang H, Ochani M, Li J, et al.: Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proc Natl Acad Sci U S A* 2004, 101:296–301.
43. Angus DC, Yang L, Kong L, et al.: Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. *Crit Care Med* 2007, 35:1061–1067.
44. Tsung A, Sahai R, Tanaka H, et al.: The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005, 201:1135–1143.
45. Qiu S, Wang H, Yuan R, et al.: Role of HMGB1 in apoptosis-mediated sepsis lethality. *J Exp Med* 2006, 203:1637–1642.
- A very important work that offers a mechanistic explanation linking apoptosis and death during sepsis.
46. Bouchon A, Facchetti F, Weigand MA, Colonna M: TREM-1 amplifies inflammation and is a crucial mediator of septic shock. *Nature* 2001, 410:1103–1107.
47. Adib-Conquy M, Goulenok M, Laurent C, et al.: Enhanced plasma levels of soluble triggering expressed on myeloid cells-1 and procalcitonin after cardiac surgery and cardiac arrest in the absence of infection. *Shock* 2007, in press.
48. Moestrup SK, Moller HJ: CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. *Ann Med* 2004, 36:347–354.
49. Imai T, Fujita T, Yamazaki Y: Beneficial effects of apolipoprotein A-I on endotoxemia. *Surg Today* 2003, 33:684–687.
50. Jacque B, Stephan K, Smirnova I, et al.: Mice expressing high levels of soluble CD14 retain LPS in the circulation and are resistant to LPS-induced lethality. *Eur J Immunol* 2006, 36:3007–3016.
51. Munford RS, Pugin J: Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *Am J Respir Crit Care Med* 2001, 163:316–321.
52. Pathan N, Hemingway CA, Alizadeh AA, et al.: Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock. *Lancet* 2004, 363:203–209.
53. Witzensbichler B, Westermann D, Knueppel S, et al.: Protective role of angiotensin-1 in endotoxic shock. *Circulation* 2005, 111:97–105.
54. Orfanos SE, Kotanidou A, Glynos C, et al.: Angiotensin-2 is increased in severe sepsis: correlation with inflammatory mediators. *Crit Care Med* 2007, 35:199–206.
55. Imai Y, Kuba K, Rao S, et al.: Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005, 436:112–116.
56. Faggioni R, Fantuzzi G, Gabay C, et al.: Leptin deficiency enhances sensitivity to endotoxin-induced lethality. *Am J Physiol* 1999, 276:R136–142.
57. Puneet P, Hegde A, Ng SW, et al.: Preprothymosin-A gene products are key mediators of lung injury in polymicrobial sepsis. *J Immunol* 2006, 176:3813–3820.
58. Deng J, Muthu K, Gamelli R, et al.: Adrenergic modulation of splenic macrophage cytokine release in polymicrobial sepsis. *Am J Physiol Cell Physiol* 2004, 287:C730–736.
59. Wong LY, Cheung BM, Li YY, Tang F: Adrenomedullin is both proinflammatory and anti-inflammatory: its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. *Endocrinology* 2005, 146:1321–1327.
60. Borovikova LV, Ivanova S, Zhang M, et al.: Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000, 405:458–462.
61. Pavlov VA, Ochani M, Gallowitsch-Puerta M, et al.: Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc Natl Acad Sci U S A* 2006, 103:5219–5223.