REVIEW ARTICLE

Inflammatory mediators in sepsis: Cytokines, chemokines, adhesion molecules and gases

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Abstract
Sepsis is a systemic inflammatory response syndrome in response to severe infection. An overwhelming systemic response brought about by the release of various inflammatory mediators can lead to shock, multiple organ damage and death. Cytokines play an important role in the pathogenesis of sepsis and are regulated by a complex network of pro- and anti-inflammatory mediators. Various chemokines sequester neutrophils into the target organ, further augmenting inflammation. Chemokine receptor antagonism represents a major therapeutic approach against sepsis. Adhesion molecules mediate the migration of leukocytes towards the site of inflammation and their activation. Gaseous mediators such as nitric oxide and hydrogen sulfide are also involved in the pathogenesis of inflammation. Our review summarizes the current understanding of the roles of various inflammatory mediators in sepsis.

Key words: Adhesion molecules, chemokines, cytokines, gaseous mediators, sepsis

Introduction
Sepsis is defined as the presence of bacteria or their toxins in blood or tissue and the systemic response that follows. A systemic inflammatory response syndrome (SIRS) triggered by infection is known as sepsis. Severe sepsis is characterized by sepsis leading to failure of at least one organ and septic shock is defined as severe sepsis accompanied by hypotension unresponsive to fluid resuscitation. Severe sepsis and septic shock constitute one of the leading causes of mortality among intensive care unit and postoperative care patients (1). The incidence of sepsis in North America has been reported to be 3.0 cases/1000 population, which translates into an annual number of 750 000 cases, 210 000 of which are fatal, and this represents a large socioeconomic burden (2,3). The incidence of mortality due to sepsis is increasing and the most likely causes are the increased incidence of resistant pathogens and the advances in medical and surgical procedures that save the lives of many patients but leave them immunocompromised and in a state in which they are highly susceptible to death from severe sepsis and septic shock (2-4).

Sepsis and the events that follow it represent stages in a progressive condition, i.e. a systemic response to infection brought about by various inflammatory mediators, such as cytokines and chemokines. A relationship between the cytokine cascades and sepsis has long been established but new evidence suggests that adhesion molecules and hydrogen sulfide also play a key role.

Pathophysiology of sepsis
Innate immunity preserves host integrity with respect to molecular components of invading microbial pathogens. These include endotoxins, such as lipopolysaccharide (LPS) from Gram-negative bacteria, peptidoglycans and flagella of Gram-negative and -positive bacteria, lipoteichoic acid from Gram-positive bacteria, glycolipids of mycobacteria, mannan from fungi and double-stranded RNA of viruses. However, sepsis is expressed as the result of overwhelming systemic
disease caused by severe infection with a microbial pathogen. There is exaggerated stimulation of the normal host responses in order to eradicate the invasive pathogens, leading to excessive release of inflammatory mediators and vasodilation. Direct bacterial invasion is the most frequently underlying infection, resulting in sepsis in which Gram-negative bacteria account for about half the cases. However, more recently, there has been a swing in the predominant infective organisms involved in sepsis from Gram-negative to -positive bacteria (2). There has been alarming growth in the incidences of fungal and virus sepsis over the last decade (2). Polymicrobial sepsis occurs frequently, and in many cases an accurate microbial diagnosis is not achieved (3).

The innate immune response is triggered by monocyte/macrophage recognition of specific microbial pathogens by a family of “pathogen-associated molecular pattern (PAMP) receptors” [toll-like receptors (TLRs)] (5–8). LPS complexes with a specific LPS-binding protein (LBP) in the plasma and then binds to a membrane receptor, CD14, on effector cells such as macrophages and endothelial cells. This initiates intracellular signal transduction via TLR4. This membrane signaling causes kinases in the cytoplasm to activate nuclear factor (NF)κB, resulting in secondary macrophage activation and an inflammatory cascade mediated by cytokine release.

Cytokines are a key element in the inflammatory response that characterizes sepsis and is regulated by the elaborate network of pro- and anti-inflammatory mediators shown in Table I (9,10). Stimulation of macrophages causes the production of large amounts of tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 (11,12). TNF-α is one of the most important cytokines involved in the pathophysiology of sepsis and is released early in the process.

Pro-inflammatory mediators facilitate inflammation by promoting endothelial cell–leukocyte adhesion, inducing the release of arachidonic acid metabolites and complement activation. In addition, pro-inflammatory mediators also promote coagulation by increasing tissue factors and membrane coagulants, inhibiting anticoagulant activity by decreasing thrombomodulin and inhibit fibrinolysis. In contrast, anti-inflammatory mediators inhibit inflammation by inhibiting TNF-α, augmenting acute-phase reactants and immunoglobulins and inhibiting T-lymphocyte and macrocyte functions (11). Anti-inflammatory mediators also inhibit activation of the coagulation system by cytokines. The anti-inflammatory response serves as a negative feedback mechanism to downregulate the synthesis of pro-inflammatory mediators and modulate their effects, thereby restoring homeostasis. SIRS is the result of an excessive pro-inflammatory response, whereas a compensatory anti-inflammatory reaction (CARS) is the result of inappropriate immunosuppression (Fig. 1) (12). If an imbalance develops between SIRS and CARS, homeostasis is violated.

**Models for the study of sepsis**

The best-known human model of sepsis is experimental human endotoxemia: volunteers are challenged by i.v. administration of a low-dose
endotoxin, which may result in a high core temperature, headache, nausea, myalgia and cardiovascular responses (13).

Many animal models have also been developed and modified to study the pathophysiology of sepsis (14). Two lethal models of sepsis have been most widely used in mice: one involves injection of LPS; the other involves performing cecal ligation and puncture (CLP) surgery (15). LPS is a stable, relatively pure compound. It is relatively easy to use; however, many clinical trials of anti-cytokines based on promising results in this model turned out to be unsuccessful. In contrast, the CLP model mimics clinical problems such as perforated appendicitis and diverticulitis; however, it is more difficult to control the magnitude of the challenge (14). It has been demonstrated (16) that LPS and CLP models have similar mortality and morbidity but there are significant differences in the kinetics and magnitude of plasma and peritoneal levels of cytokines (TNF-α, IL-1β, IL-6) and chemokines [keratinocyte-derived chemokine (KC), macrophage inflammatory protein (MIP)-2].

Fig. 1. Sepsis cascade illustrating local inflammatory and anti-inflammatory responses through systemic spillover of mediators leading to CHAOS.
Role of cytokines in sepsis

Activation of inflammatory pathways, which involves cytokines, is considered to play a crucial role in the pathogenesis of sepsis. Cytokines are a group of soluble, low-molecular-weight glycoproteins produced by a large variety of cells that are thought to be important for host defense, wound healing and other essential host functions. Although cytokines are important in homeostasis, excessive production and release of cytokines can initiate widespread tissue injury, which can often lead to organ damage. Sepsis syndrome, which results from the systemic inflammatory response to infection, is caused by overwhelming release of cytokines into the systemic circulation.

Normally, cytokines are not stored in intracellular compartments but are synthesized and released in response to tissue and cell damage. This regulation of cytokine production and release occurs predominantly at the level of gene transcription, with new cytokine mRNA expression. One important transcription factor is NFxK, which consists of homodimers and heterodimers of proteins of the Rel family. NFxK, which exists as a p50–p65 heterodimer, appears to play a central role in regulating the cytokine response. NFxK exists in an inactive form in the cytoplasm, where it is bound to the inhibitory IxB proteins. It regulates the transcription of various pro-inflammatory and immunoregulatory cytokines, genes encoding for immunoreceptors, cell adhesion molecules, hematopoietic growth factors, acute-phase proteins and enzymes such as cyclooxygenase-2 and inducible nitric oxide (NO) synthase (17). NFxK activation is important for the transcription of many cytokines and related molecules, such as TNF-α and IL-1β, -6 and -8 (18).

The cytokine response is regulated by an intricate network of pro- and anti-inflammatory mediators (16). Cytokines are released in a sequential manner, resulting in a “cytokine cascade”. This is initiated when the pathogenic organisms are exposed to the host immune response, which induces the production and secretion of “early” cytokines such as TNF-α and IL-1β. These act synergistically to stimulate the release of “later” cytokines such as IL-6 and -8 and anti-inflammatory cytokines, such as IL-4, -10 and -13 (19).

Pro-inflammatory cytokines

TNF-α

TNF-α is a 17-kDa protein produced primarily by monocytes, macrophages, lymphocytes, neutrophil granulocytes, mast cells and fibroblasts in response to inflammation. It acts via specific cell membrane-bound receptors (20). Injection of TNF-α into experimental animals causes a syndrome indistinguishable from septic shock. Circulating levels of TNF-α are elevated in a number of conditions that lead to shock and it is now thought that TNF-α is one of the major mediators of shock (20,21).

In sepsis, TNF-α and IL-1β are released during the first 30–90 min after exposure to LPS and in turn activate a second level of inflammatory cascades including cytokines, lipid mediators and reactive oxygen species, as well as upregulating cell adhesion molecules that result in the initiation of inflammatory cell migration into tissues (22).

Animal studies (23) have demonstrated that the concentration of TNF-α in plasma is significantly increased in severe sepsis and in animals that subsequently died. Infusion of neutralizing antibodies to TNF-α can improve survival in animal models of septic shock (24,25). Similarly, in humans, TNF-α levels in plasma are raised in patients with SIRS. These levels increase substantially in severe sepsis and in patients who subsequently die (26–28).

TNF-α is a primary mediator of inflammation and an inducer of the pathophysiological problems associated with sepsis. Experiments conducted with anti-TNF-α antibodies indicated that blocking TNF-α in bacterial or endotoxin-induced shock models led to a dramatic decrease in the levels of other cytokines measured in the bloodstream. In baboons infused with live Escherichia coli, anti-TNF-α almost abolished the levels of circulating IL-1β, -6 and -8 (29,30). However, some studies (31,32) have also shown that antibodies against TNF are either ineffective or worsen the outcome in a standardized model of peritonitis-induced severe sepsis. Several therapeutic agents that target TNF have been tested in clinical trials of sepsis, but no significant survival advantage has been observed. One confounding factor is that TNF levels are not usually increased in enrolled patients, partly because of the rapid kinetics of the TNF response (33,34). The combined insights obtained from the failure of clinical trials of anti-TNF strategies (35,36), as well as observations related to the kinetics of TNF released compared with the onset of lethality, prompted a search for a mediator of sepsis (37). This led to the identification of high mobility group box 1 (HMGB1) as a product of stimulated macrophages which is released significantly later than TNF (38–40).

HMGB1

HMGB1 is a 30-kDa cellular protein that was discovered 30 years ago. It was first co-purified from nuclei with histones and termed “high mobility group 1” (HMG-1) protein because of its rapid
mobility on electrophoresis gels (41). It has subsequently been renamed HMGB1. Originally it was described as a nuclear DNA-binding protein; however, more recent findings have indicated that HMGB1 is active in DNA recombination, repair and replication and gene transcription, facilitated by internal repeats of positively charged domains of the N terminus (HMG boxes) (42). The presence of inflammatory stimuli such as LPS leads to the production of HMGB1 from macrophages in the later stages of sepsis. HMGB1 binds to the receptor for advanced glycation end products (RAGE) on endothelial cells and induces intracellular signaling through kinases (Jun N-terminal kinase (JNK), extracellular signal-regulated kinase 1/2 and p38 MAP/microtubule affinity regulating kinase (MARK)). This leads to nuclear translocation of transcription factors (NFxB and Specificity protein-1 (SP-1)) (43–46). In response to HMGB1 stimulation, endothelial cells express RAGE, adhesion molecules [vascular cell adhesion molecule-1 (VCAM-1) and interstitial cell adhesion molecule (ICAM-1)], pro-inflammatory cytokines, including TNF-α and IL-8, and chemokines such as monocyte chemotactic protein (MCP)-1, plasminogen activator inhibitor (PAI)-1 and tissue plasminogen activator (47). In enterocytes, HMGB1 increases the activity of intrinsic NO synthase, leading to heightened NO production and increased permeability, which results in enhanced bacterial translocation through the gut barrier (46,48).

Studies carried out in a murine model of sepsis (i.e. CLP) have shown that there is significant elevation of serum HMGB1, beginning 18 h after induction of peritonitis (49). HMGB1 remained significantly high for at least 72 h, a time course similar to the delayed kinetics observed in endotoxemia (49,50). The late appearance of HMGB1, corresponding to the onset of death as a result of sepsis, distinguishes it from TNF and other early-acting mediators of systemic inflammatory responses (51).

In clinical studies, patients with sepsis-induced organ dysfunction (i.e. hypotension, hypoxemia, lactic acidosis and disseminated intravascular coagulation) have higher than normal serum levels of HMGB1 (50). Furthermore, septic patients who died had significantly higher serum HMGB1 levels in comparison with survivors (50).

More recently (49), treatment with anti-HMGB1 antibodies beginning as late as 24 h after CLP surgery was shown to significantly increase survival (72% vs 28% for non-immune IgG-treated controls; n = 18 mice in each group; p < 0.03 by Fisher's exact test). More importantly, administration of anti-HMGB1 antibodies at this late stage “rescued” animals that were already exhibiting signs of severe sequelae of sepsis. In addition, anti-HMGB1 antibodies do not significantly change bacterial counts in the spleens of infected septic animals; this indicates that the survival advantage conferred by anti-HMGB1 antibodies is not due to inhibition of bacterial proliferation (49).

Studies have also shown (52) that ethyl pyruvate, a non-toxic food additive, which inhibits HMGB1 production in vivo, improves survival in a sepsis model in mice when administered 24 h after onset of sepsis. These results make HMGB1 a promising candidate as a target for the treatment of septic patients, particularly because such patients also show elevated serum concentrations of HMGB1 (50). Similar results were obtained when administering the peptide A box, an antagonist to HMGB1 that attenuates the release of TNF and IL-1β from macrophages, and significantly protects animals against endotoxin-induced lethality. Moreover, administration of A box rescued mice from the lethal effects of sepsis, even when treatment was initiated as late as 24 h after cecal perforation (49). Hence, HMGB1 is a mediator of sepsis, and blocking endogenous HMGB1 with either antibodies or specific HMGB1 antagonists (A box or ethyl pyruvate) may offer a possible future intervention for patients with sepsis. Anti-HMGB1 therapies have a significantly wider therapeutic window than TNF-targeted interventions, and it may be possible to develop inhibitors of HMGB1 for the treatment of sepsis (53).

**IL-1**

IL-1 and TNF-α share a remarkable array of biological effects. Administration of high doses of either TNF-α or IL-1 to laboratory animals reproduces many characteristics of sepsis syndrome (54,55). The simultaneous administration of TNF-α and IL-1 results in synergistic toxicity in rabbits (55). IL-1β is a potent pro-inflammatory cytokine which, like TNF-α, is derived predominantly from macrophages. It activates neutrophils, induces upregulation of adhesion molecules on both leukocytes and endothelium and induces a shock-like state in animal models (54). It activates the production of other cytokines, including IL-6 and -8 and TNF-α. Like TNF-α, IL-1β appears to be a predictor of the severity of sepsis.

**IL-6**

IL-6 is produced by a wide range of cells, including monocytes/macrophages, endothelial cells, fibroblasts and smooth muscle cells in response to stimulation by endotoxin, IL-1β and TNF-α
IL-10 is a non-covalently linked homodimeric cytokine (160 amino-acid protein) that is produced by a large variety of cells, including monocytes, macrophages, B and T lymphocytes and natural killer (NK) cells.

IL-10 is an anti-inflammatory cytokine (63,64). The inhibitory effects of IL-10 on TNF-α and IL-1 production are crucial to its anti-inflammatory activities because these two cytokines often have synergistic effects on the inflammatory response.

IL-10 plasma levels are elevated in animal models of endotoxemia. IL-10 inhibits the release of pro-inflammatory cytokines (e.g. IL-1β, IL-6 and TNF-α) from monocytes/macrophages, thus preventing subsequent tissue damage (63–65). Moreover, IL-10 also stimulates production of the naturally occurring IL-1 receptor antagonist (IL-1Ra) and release of the soluble p75 TNF receptor (66). Administration of IL-10 has a protective effect in animal models of sepsis. Treatment with murine IL-10 protected BALB/c mice from a lethal intraperitoneal injection of endotoxin. These data implicated IL-10 as a candidate for the treatment of bacterial sepsis and more generally as an effective anti-inflammatory reagent (65).

### Soluble receptors and receptor antagonists

#### Soluble TNF-α receptors

Soluble TNF-α receptors (sTNFRs) are the most intensely studied soluble cytokine receptors in sepsis. Two soluble receptors have been described, sTNFR-p55 and -p57, which are produced by proteolytic cleavage of the extracellular binding domain of the TNF receptors from the cell surface. These receptors modulate the actions of TNF-α by binding to the cytokine.

Plasma sTNFR concentrations are excessively increased in the circulation of patients with endotoxemia (19). In human sepsis, higher plasma concentrations of sTNFRs were detected in patients with severe sepsis, and correlated with higher mortality (67).

#### IL-1Ra

IL-1Ra is a member of the IL-1 family. It blocks IL-1 activity by competitive binding to IL-1 receptors without inducing any intracellular response (68). IL-1Ra was shown to attenuate endotoxin effects in animal models of sepsis. Ohlsson et al. (69) reported that IL-1Ra reduced the lethality of endotoxin-induced shock in rabbits. Fisher et al. (70) demonstrated that IL-1Ra significantly attenuated the cytokine cascade and improved survival in E. coli septic shock in baboons. In septic patients, a higher plasma concentration of IL-1Ra is associated with the more severe forms of sepsis (71). However, its functional role in sepsis is still uncertain.

### Role of chemokines and their receptors in sepsis

The host response to pathogens in sepsis is characterized by infiltration of specific leukocyte populations, including neutrophils and monocytes, into host tissues. It is known that this process is predominantly mediated by a family of cytokines: chemokines (72). While production of chemokines is essential for host defense against bacteria, overproduction of these mediators has been shown to play an important role in the pathogenesis of sepsis. The sequestered over-activated inflammatory leukocytes, in combination with their overproduction of pro-inflammatory mediators and reactive oxygen species, are believed to cause the tissue damage and the subsequent multiple organ failure and death associated with sepsis.

Chemokines are small (8–10 kDa) proteins. Over 40 chemokines have been identified to date. They can be subdivided into four families based on the relative position of cysteine residues (73,74). Two major subfamilies, CXC and CC chemokines, have been extensively investigated in sepsis. In the CXC chemokines, the first two cysteine residues are separated by a single amino acid, while in the CC chemokines, the first two cysteine residues are adjacent to each other. Chemokines bind to a family of seven-transmembrane-domain G protein-coupled receptors on the surface of leukocytes (73). Nearly 20 different types of receptors have been described.
but only a few are known to be involved in the acute inflammatory response during sepsis.

CXCR1 and CXCR2 are important chemokine receptors responsible for the activation of neutrophils, endothelium, epithelium, macrophages and other cells (74). In humans, systemic administration of endotoxin in healthy volunteers leads to a significantly elevated IL-8 level (75); however, expression of CXCR2, but not CXCR1, is reported to be reduced on neutrophils isolated from patients with septic shock compared with healthy controls (76). In mouse KC, mouse IL-8 homologs and MIP-2 have been reported to share a common receptor, CXCR2, and were found to be elevated in the lungs and plasma after hemorrhage and sepsis (77). Mice that are deficient in CXCR2 or have been treated with CXCR2-specific antibodies are protected from developing sepsis (78). Blockade of CXCR2 by anti-leukocyte, a hexapeptide inhibitor of the CXC chemokine receptor, significantly reduced polymorphonuclear influx and the lung tissue content of IL-6, KC and MIP-2 and increased tissue IL-10 levels compared with a vehicle-treated group (77). Recent work (79) has also shown that pepducins derived from intracellular loops of CXCR1 and CXCR2 prevent the IL-8 response of both receptors and reverse the lethal consequences of sepsis, including disseminated intravascular coagulation and multi-organ failure, in mice.

CCR1 is expressed by a broad spectrum of leukocytes, including neutrophils, monocytes, eosinophils and lymphocytes (74). CCR1 has three main ligands in vivo: CCL3 (MIP1-α), CCL5 [regulated on activation, normal T cell expressed and secreted (RANTES)] and CCL6 (chemokine C10) (80). MIP1-α and chemokine C10 have been demonstrated to play protective roles against sepsis in a murine CLP model: MIP1-α enhanced the protective innate immune response against sepsis by activating macrophages but not neutrophils, while chemokine C10 displayed a therapeutically potent yet focused enhancement of inflammatory parameters, preventing the exacerbation of systemic inflammation (81,82). In contrast, RANTES has been identified as acting in a CCR1-dependent manner to trigger the overproduction of pro-inflammatory cytokines and chemokines, resulting in aggravated injury and mortality following sepsis (80). CCR1 knockout mice are significantly protected against CLP-induced lethality by promoting cytokine production and enhancing macrophage activity (80). Although CCR1 deficiency had no effect on inflammatory cell recruitment to the peritoneal cavity, it has been reported (80,83) that blockage of MIP-1α by antibodies attenuates myeloperoxidase (MPO) activity in the lungs of CLP mice.

CCL2 (MCP-1) has been shown to bind predominantly to the CC chemokine receptor 2 (CCR2) (84). In addition to binding MCP-1, CCR2 also works as a receptor for three other chemokines: MCP-3, -4 and -5 (85). In mouse CLP sepsis models, it has been demonstrated (86) that MCP-1 levels are greatly elevated in certain tissues, including the lung, liver and kidney. It is known (74) that CCR2 is mainly expressed on monocytes and activated T cells. However, it has been reported (83) that, 6 h after CLP, expression of CCR2 mRNA on neutrophils is significantly upregulated. In either CCR2 knockout mice or wild-type mice treated with antibody against MCP-1, MPO activity in the lung is significantly decreased in a CLP model (83). However, CCR2 blockade by antibodies increases local and systemic IL-10 levels and causes impaired bacterial clearance and increased kidney injury in mice (87). Therefore, while CCL2 and CCR2 may contribute to the pathology of sepsis by leukocyte recruitment and activation, inhibition of CCR2 or its ligands in sepsis could possibly result in greater mortality.

Another possible therapeutic target for sepsis is CCR4. CCR4 knockout mice have exhibited significantly decreased mortality on administration of high- or low-dose bacterial LPS compared with wild-type mice (88).

Despite the complexity and redundancy of the chemokine system, all these studies indicate that chemokines and their receptors play a critical role in the pathogenesis of sepsis. More and more reports of chemokine receptor antagonists have been published in the past few years. These antagonists have been tested both in vitro and in animal disease models. We hope that the development of this field can finally lead to clinically effective drugs for sepsis in the near future.

Adhesion molecules in sepsis

Sepsis is associated with widespread vascular endothelial activation, with increased expression or release of endothelial adhesion molecules (89). Selectins, ICAM-1 and VCAM-1 are among the most important endothelial adhesion molecules involved in various inflammatory responses and sepsis.

Selectins

Selectins are a family of glycoproteins whose primary role is to mediate the initial step in leukocyte migration of loose adherence and rolling on activated endothelium. The selectin family is composed of three members named according to the
cells in which they were originally discovered: E- (endothelium) selectin; P- (platelet) selectin; and L- (leukocyte) selectin (90).

E-selectin (CD62E) is a 115-kDa endothelial transmembrane glycoprotein. Its expression is normally not detected in resting endothelium, but is strongly and rapidly induced by inflammatory cytokines, including TNF-α, IL-1β and LPS (91). P-selectin (CD62P) is expressed on platelets and endothelial cells and stored in α-granules and Wiebel–Palade bodies, respectively (92). Similarly to E-selectin, P-selectin expression is induced by inflammatory responses. L-selectin (CD62L) is expressed essentially on all blood leukocytes.

L- and P-selectin mediate the initial capture of leukocytes from the streaming blood, while the synergistic action of L- and either E- or P-selectin is required for optimal and stable leukocyte rolling (93). The observation that L-selectin-deficient mice were resistant to LPS-induced mortality suggested the involvement of L-selectin in septic shock (94). Donnelly et al. (95) found that patients with high levels of soluble L-selectin are resistant to acute respiratory distress syndrome (ARDS). Thus, it was proposed that L-selectin may have a dual role: first as an adhesion molecule involved in leukocyte rolling and second as a signaling receptor for LPS (96,97). P-selectin was later found to be able to bind bacterial LPS (96).

ICAM-1 and VCAM-1

ICAM-1 (CD54) is an inducible cell adhesion glycoprotein belonging to the immunoglobulin supergene family which is expressed on the surface of leukocytes and endothelial cells as well as a wide variety of other cell types. ICAM-1 is constitutively present on the cell surface and is upregulated in response to a variety of inflammatory mediators (98). Interactions of ICAM-1 with the two β2 integrins, CD11a/CD18 (lymphocyte function antigen-1 (LFA-1)) and CD11b/CD18 (modulator of apoptosis-1 (MAC-1)), on the surface of leukocytes are critical for the trans-endothelial migration of leukocytes to sites of inflammation (99). VCAM-1 is a 110-kDa member of the immunoglobulin supergene family. Similar to ICAM-1, VCAM-1 is expressed on a wide variety of other cell types, including leukocytes and endothelial cells. The ligand for VCAM-1, very late antigen 4, is present on lymphocytes, monocytes and granulocytes, including neutrophils (100).

ICAM-1 and VCAM-1 are involved in mediating firm adhesion between leukocytes and endothelium and subsequent diapedesis during inflammation. Levels of ICAM-1 and VCAM-1 are upregulated in endothelial cells by inflammatory mediators such as the cytokines TNF-α, IL-1β and interferon (IFN)-γ, and bacterial LPS. Clinical studies (101,102) have shown that increased plasma concentrations of ICAM-1 and VCAM-1 are predictive of the development of multiple organ dysfunction syndrome and death in neonatal and adult sepsis. Furthermore, ICAM-1-deficient mice show leukocytosis and resistance to septic shock (103). Blockade of adhesion molecules reduces the severity of experimentally induced sepsis, underscoring their importance in endothelium–leukocyte interactions during sepsis and multiple organ dysfunction syndrome (102).

Gaseous mediators in sepsis

Gaseous mediators such as NO and hydrogen sulfide (H2S) comprise a unique class of biomolecules that is indispensable for maintaining the homeostasis of biological systems and exerting both harmful and beneficial effects at cellular and vascular levels in a wide range of diseases. Sepsis is one condition in which gaseous mediators are considered to play a key role in pathogenesis.

NO

NO is synthesized by at least three distinct isoforms of NO synthase (NOS): endothelial NOS (eNOS); inducible NOS (iNOS); and neuronal NOS (nNOS). Endothelium-derived NO is physiologically important for vascular homeostasis, keeping the vasculature relaxed, preventing platelet aggregation in the intima and leukocyte adhesion and inhibiting smooth muscle proliferation.

In various LPS and CLP models of sepsis, rapid but transient increases in iNOS and nNOS mRNA expression have been detected, followed by or commensurate with increased NOS activity and elevated levels of NO-oxidized metabolites in different tissues and plasma (104–110). During the progression of sepsis, various inflammatory mediators, particularly the pro-inflammatory cytokines TNF and IL-1, either alone or synergistically, have been implicated in the induction and activation of iNOS (111) throughout the organs (112) and of nNOS in brain and skeletal muscle (113). Endotoxin has also been shown to increase the constitutive release of NO by the endothelium (114) and the activity of iNOS in septic shock (115). In addition to endotoxin, cell-wall components and enterotoxin from Gram-positive organisms are also able to stimulate NO release (116).

Under septic conditions, overproduction of NO by upregulation of iNOS and nNOS in endothelial
and muscle cells may contribute significantly to hemodynamic alterations such as hypotension after LPS administration (117), endotoxin- (114) and TNF-induced (118) vasodilation and vascular unresponsiveness to vasoconstrictors. Mice lacking iNOS have been reported to be resistant to endotoxin-induced mortality (119) and vascular hypocontractility (120), supporting a key role for iNOS in endotoxic shock. Similarly, in a rat CLP model of sepsis (113), microvascular reactivity to acetylcholine, quantified as changes in arteriolar diameter and downstream capillary red blood cell velocity, has been shown to be impaired in skeletal muscle by NO and restored by nNOS inhibition. NO blockage in CLP rats is also able to reverse arteriolar hyporesponsiveness to catecholamines and endothelin in cremaster muscle, as measured by changes in arteriolar contraction (121,122). Moreover, excessive NO production is an important mediator of cardiac dysfunction and also contributes to multiple organ dysfunction in sepsis. Myocardial iNOS activity has been reported in response to endotoxin and cytokines and is inversely correlated with myocardial contractile performance (123). Endotoxin has been shown to induce iNOS synthesis in human intestinal and liver cells (124) and to cause morphologic and functional damage, as evidenced by increases in intestinal epithelial permeability (125) and, in animals, bacterial translocation and elevation of plasma liver enzymes. In addition to acting as a vasodilator, NO has a downregulating effect on TNF and IPN production in response to staphylococcal enterotoxin in mice (126).

**H2S**

H2S has been well known for a long time as a toxic gas with the smell of rotten eggs. Recent studies (127) have shown that H2S is endogenously synthesized by pyridoxal 5'-phosphate-dependent enzymes, cystathionine beta-synthase (CBS) or cystathionine gamma-lyase (CSE). CBS and CSE are the main H2S-producing enzymes in the brain and cardiovascular system, respectively. Recently (128–130), endogenous H2S has been proposed as a physiologically active gasotransmitter, which functions as a novel vasodilator and neuromodulator. By acting on adenosine triphosphate (ATP)-dependent potassium channels, endogenous H2S can hyperpolarize cell membranes, relax smooth muscle cells and thus regulate blood pressure in various physiological and pathological conditions. Furthermore, H2S can promote hippocampal long-term potentiation and contribute to some nervous system diseases (131–133).

Notably, the most recent studies have shown that endogenous H2S plays a potential pro-inflammatory role in several inflammatory conditions. LPS-induced endotoxemia resulted in a significant increase in the plasma H2S concentration and in H2S formation in both the liver and kidneys (134,135). At the same time, pronounced upregulation of CSE mRNA was detected in the same organs. Prophylactic as well as therapeutic administrations of DL-propargylglycine (PAG), an inhibitor of CSE, exert a noticeable anti-inflammatory activity, as evidenced by reduced MPO activity in the lung and liver (134,135). As a result, inhibition of H2S synthesis mitigates the endotoxia-associated circulatory failure (hypotension and tachycardia), hepatic injury, pancreatic damage and neuromuscular injury (135). Similarly, the H2S contents of different artery tissues were significantly increased in both endotoxic shock and CLP-induced septic shock (136). The endogenous level of H2S generated by the artery tissues is negatively related to the hemodynamic parameters in septic and endotoxic shock (136). Moreover, in carrageenan-induced hindpaw edema, H2S-synthesizing enzyme activity in the hindpaw was enhanced. Pretreatment with PAG markedly reduced hindpaw edema and MPO activity in the hindpaw (137). Induction of acute pancreatitis in mice by hyperstimulation with a dose of caerulein elevated the plasma H2S level, whereas blocking the activity of CSE significantly reduced inflammatory cell infiltration into both the pancreas and lung, thereby reduced the severity of pancreatitis and associated lung injury (138). Therefore, H2S exhibits a pro-inflammatory activity in some inflammatory diseases and plays a possible role in regulating the severity of multiple organ dysfunction.

As H2S plays an essential role in regulating cardiovascular function, inhibition of H2S formation affords protection against myocardial and lung injury. Both in vitro and in vivo studies (139) showed that H2S has a negative inotropic effect on cardiac function, and this effect could be partly abolished by K+ ATP channel blocker. Isoproterenol-induced myocardial injury resulted in decreases in myocardial and plasma H2S levels and CSE activity as well as elevation of CSE gene expression. As a result of its direct scavenging of oxygen free radicals and reduction of the accumulation of lipid peroxidations, exogenous administration of the H2S donor NaHS reduced the mortality rate and isoproterenol-associated decrease in myocardial contractile function and attenuated subendocardial necrosis, capillary dilatation, leukocytic infiltration, fibroblast swelling and fibroblastic hyperplasia (140). These effects appear to be mediated by the scavenging of oxygen free radicals by H2S. In contrast, hypoxic pulmonary hypertension suppressed H2S formation, and the activity and gene
expression of CSE. Exogenous application of H₂S improved pulmonary arterial hypertension, pulmonary vascular structure remodeling and ultrastructural changes of the pulmonary small arteries (141).

What next?
Recent studies have shown that there has been substantial progress in understanding the role of inflammatory pathways in the pathogenesis of sepsis. The ultimate severity of the disease greatly depends on interplay between pro- and anti-inflammatory mediators and repair mechanisms, which start soon after the initiation of lung injury. We hope that clinical trials of new anti-inflammatory pharmacological strategies will reduce the morbidity and mortality of this common clinical condition.

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Inflammatory mediators in sepsis


