Cellular Mechanisms in Sepsis

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Mortality remains very high among septic patients despite the advanced treatments rendered in intensive care units. The development of septic shock is multifactorial. Tissue damage and organ dysfunction may be caused not only by the microorganisms but also by the inflammatory mediators released in response to the infection. Cytokines (tumor necrosis factor, interleukin-1, interleukin-6, interleukin-8, high-mobility group box-1 protein, macrophage migratory inhibitory factor) and noncytokines (nitric oxide, platelet-activating factor, complements, and eicosonoids) may inflict tissue injury and contribute to multiple organ dysfunction and cell death (or apoptosis). Gram-negative bacteria are the most common organisms identified in septic patients. The pathological effects of gram-negative bacteria are conveyed through lipopolysaccharide derived from the bacterial cell membrane. Lipopolysaccharide activates the nuclear factor κ B, which triggers the release of inflammatory mediators. Protein components from gram-positive bacteria, fungi, or viruses may evoke the activation of nuclear factor κ B in a similar fashion as lipopolysaccharide. Endogenous anti-inflammatory mediators are released in response to the infection and act to control the overwhelming systemic inflammatory response. The fragile balance between negative and positive feedback on the inflammatory mediators is the key factor that modulates the cellular damage and influences the clinical outcome.

Key words: *cellular, sepsis*

Sepsis exacts a devastating toll among critically ill patients. It is the most common cause of death in intensive care units. It is estimated that every year, some 215 000 fatalities are attributed to sepsis alone in the United States [1]. Despite the development of new antibiotics and aggressive vasopressive drug therapy, mortality has remained unacceptably high over the past 3 decades and ranges between 30% and 50% in patients with severe sepsis, up to 70% when a state of shock is present [2,3].

A better understanding of the pathophysiology and cellular mechanisms in sepsis is indispensable to the assimilation of the biochemical alterations in the host tissues and the formulation of the appropriate management.

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Pathogenesis of Sepsis

The pathophysiology of sepsis involves the initiation of intricate reactions triggered by the microbial pathogens. Gram-negative bacteria are the most commonly isolated in septic patients [4]. Endotoxin is a compound molecule derived from gram-negative bacteria membrane and is the initial stimulus to cellular reactions leading to the release of cytokines [5]. Other microorganisms (gram-positive bacteria, virus, or fungus) may induce inflammatory responses and a state of shock similar to gram-negative infections [6-8]. Two distinct mechanisms contribute to the hemodynamic collapse in sepsis: (1) extrinsic: toxins, endotoxin, and protein particles from gram-positive bacteria, viruses, or fungi, and (2) intrinsic (or endogenous): proinflammatory mediators released by the host immune cells (Figure 1).

Extrinsic Factors

Endotoxin

Endotoxin is a lipopolysaccharide (LPS) and contains 3 different parts: (1) the innermost part or the lipid A, considered to be the spearhead or toxic portion of the molecule; (2) the O-polysaccharide, a variable side chain situated at the outer surface of the endotoxin molecule with changing configuration among gram-negative bacteria; and (3) a third portion, also called core region, linking the inner and outer portions. Lipid A and the core region have a more conserved structure with a molecular resemblance among most gram-negative bacteria [9]. Monoclonal antibodies against lipid A and the core region have been genetically manufactured and tried in clinical research [10,11].

Endotoxin can directly activate the macrophages, endothelial cells, and complement, leading to the liberation of various proinflammatory mediators: tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), high-mobility group box-1 protein (HMGB-1),

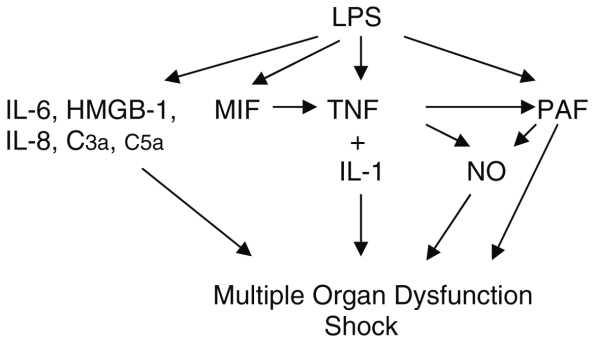

Fig 1. Activation and interaction of inflammatory mediators in sepsis.
LPS = lipopolysaccharide; IL = interleukin; MIF = macrophage migratory inhibitory factor; TNFα = tumor necrosis factor α; PAF = platelet-activating factor; NO = nitric oxide.

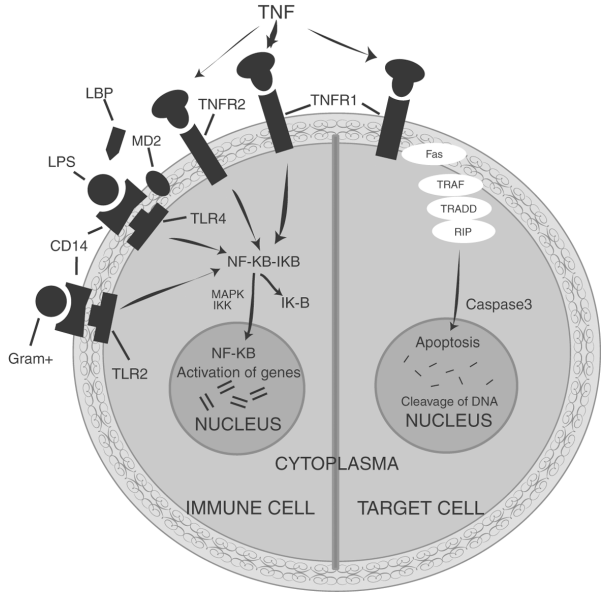

Fig 2. Cellular responses to infective agents and TNF in the immune and target cells.

macrophage migratory inhibitory factor (MIF), platelet-activating factor (PAF), nitric oxide (NO), complements, and eicosonoids [4,12] (Figure 1). LPS binds to the surface molecule CD14, present in macrophages, monocytes, and neutrophils. This coupling is facilitated by LPS-binding protein [13]. Since CD14 lacks an endoplasmic domain, the signaling is relayed through a transmembrane protein, identified as a toll-like receptor (TLR; Figure 2). The IL-1 receptor and TLR share the same endoplasmic domain and exert bactericidal and apoptotic properties. In the human genome, 10 different types of TLRs have been identified [14].

TLR type 4 (TLR4) is responsible for the recognition of most gram-negative bacteria. The binding of LPS to TLR4 is enhanced by an accessory protein, MD2, but the exact mechanism of action of MD2 is not fully understood [15]. LPS from other gram-negative bacteria, such as *Neisseria meningitides*, *Leptospira interrogans*, and *Porphyromonas gingivalis*, bind preferentially to TLR2 [14]. Signals from the CD14/TLR complex activate the nuclear factor κ B (NF-κB) through a series of phosphorylation cascades triggered by the mitogen-activated protein kinase (MAPK) family. MAPK consists of 3 major members: extracellular signal-regulated kinase, p38, and c-jun N-terminal kinase (JNK). Each one of these compounds can be activated by LPS through phosphorylation of threonine and tyrosine residues. The sequential actions of various kinases extend from the extracellular matrix to the NF-κB. Under normal conditions, NF-κB is confined to the cytoplasma in an inactive form, bound to inhibitory κ B (IK-B). The release of NF-κB is induced by the phosphorylation of IK-B by inhibitory IK-B kinase). The nuclear translocation of NF-κB promotes the production of cytokines by the target genes (Figure 2). MAPK, especially p38, plays a crucial role in the release of specific cytokines and apoptosis [16].

Gram-Positive Bacteria

Gram-positive bacteria may cause septic shock that is indistinguishable from gram-negative bacteria. Proteins from gram-positive bacteria, such as lipoteichoic acid and peptidoglycan, bind to CD14 and provoke transmembrane signaling through TLR2 (Figure 2). NF-κB is liberated in a similar fashion to LPS stimulation [14,17]. Some of the exotoxins secreted by gram-positive bacteria may contribute to a state of shock; a clear example is toxic shock syndrome, caused by *Staphylococcus aureus* [18].

Virus

Injuries inflicted to the tissues by viruses can be categorized into 3 different mechanisms: (1) the direct lytic effects on the host cells as a result of the viral replication, (2) lysis to distant organ cells from the activated complement cascade due to virus-antibody reaction, and (3) the action of circulating proinflammatory cytokines released as a provocative response to the viral infection [19]. Viral particles also bind to membrane CD14 and can evoke the activation of NF-kB through TLR signaling. TLR3 responds to viral double-stranded RNA, whereas TLR4 acts as a receptor for respiratory syncytial virus protein F [14]. Viral toxins have not shown to cause direct cellular damage, and their role in the genesis of shock has not been established.

Table 1. List of Principal Pro- and Anti-inflammatory Mediators

	Actions
Proinflammatory mediators	
TNFα	Fever, hypotension, shocklike syndrome, activation of PMN and endothelial cells
IL-1	Fever, hypotension, anorexia, sleep, T-cell and macrophage activation
IL-6	Acute-phase protein production, T-cell and B-cell proliferation
IL-8	Chemotactic for neutrophils and T cells
HMGB-1	Sepsis-like syndrome, hypotension, shock
MIF	Enhancement of TNFα and TLR4 expression
NO	Smooth muscle relaxation, oxydant cytoxic
PAF	Histamine release from platelets, activation of endothelial cells and platelets
C3a-C5a	Histamine release, increased capillary permeability, vasodilation
PGE2, PGI2	Vasolilation
TXA2	Increased pulmonary resistance
LTC4, LTD4, LTE4	Increased pulmonary capillary permeability, bronchospasm
Anti-inflammatory mediators	
IL-10	Suppression of INF, IL-1, and macrophage functions
PGI2	Down-regulation of TNFα
Soluble TNFα receptors	Blockage of TNFα receptors
IL-1 receptor antagonists	Competitive binding to IL-1 receptors, blocking the action of IL-1
Heat shock proteins	Enhanced expression of IK-B gene, negative feedback on NF-κB activation, inhibition of TNFα and IL-1
Phosphatases	Dephosphorylation of cytoplasmic substrates, reduced NF-κB-dependent TNFα production, deactivation of leukocytes and endothelial cells
Cortisol	Inhibition of NF-κB, reduction in production of TNFα, IL-1, IL-6, eicosanoids, NO, liberation of heat shock proteins

TNF = tumor necrosis factor; PMN = polymorphonuclear; IL = interleukin; HMGB-1 = high-mobility group box-1 protein; MIF = macrophage migratory inhibitory factor; TLR = toll-like receptor; NO = nitric oxide; PAF = platelet-activating factor; INF = interferon; PGI = prostacyclin; TXA = thromboxane; LTC = leukotriene C; LTD = leukotriene D; LTE = leukotriene E; IK-B = inhibitory κ B; NF-κB

Fungus

The disseminated form of fungal infection can initiate the cytokine-releasing cascade, leading to a state of shock. Like LPS, fungal proteins can activate the macrophages, the endothelial cells, and the complements. Fungal particles from *Cryptococcus neoformans* and *Aspergillus fumigatus* interact with TLR4 and induce the production of proinflammatory cytokines via the NF-κB pathway. Toxins have not been found to contribute to the state of shock in fungemia. Immunocompromised patients are particularly susceptible to complications of fungal infections [14,20].

Intrinsic Factors

External stimuli, such as severe infections, burns, trauma, and hemorrhage, can activate the immune cells and unleash a systemic inflammatory response expressed by the release of cytokines (TNFα, IL-1, IL-6, IL-8, HMGB-1, and MIF) and noncytokine mediators (NO, PAF, eicosanoids, and complements; Table 1). Among the mediators released in severe sepsis, TNFα and IL-1 are the principal cytokines implicated in the circulatory collapse in sepsis [21].

TNF

Activated macrophages secrete large amounts of TNF, and the severity of shock correlates with the TNF plasma level. TNF, formerly called cachectin, is expressed in 2 different molecules: TNFα and TNFβ. While TNFα is an important mediator of shock and multiorgan dysfunction, TNFβ (also called lymphotoxin-α) stimulates granulocyte activity and β-cell proliferation. TNFα produces a septic shock–like syndrome with hypotension and multiple organ dysfunction when injected into experimental animals [4]. TNFα induces a transmembrane signaling through TNF receptors, which have extracellular and endoplasmic domains. Two types of TNF receptors are identified: TNF-R1 and TNF-R2. TNF-R1 is present on most cells, whereas TNF-R2 is found primarily on the membrane of immune cells. Activation of NF-κB by these receptors is facilitated by TNF-associated factor. The binding of TNFα to TNF-R1 leads to programmed cell death, or apoptosis, in target cells.

TNF-R1 interacts with the Fas receptor, which favors the formation of molecular complexes made of TNF-R1–associated death domain protein, Fas-associated death domain protein, and receptor-interacting protein (Figure 2). The final step of this process is catalyzed by caspase 3, which causes the cleavage and degradation of nuclear protein substrates and apoptosis. TNF-induced gene activation includes the production of complement components, NO-synthase, cell adhesion molecules, PAF, IL-1, IL-6, IL-8, and IL-10 [22,23].

IL-1

IL-1 is secreted by monocytes, macrophages, lymphocytes, astrocytes, and endothelial cells. It produces fever, anorexia, sleep, neutrophilia or neutropenia (depending of the dose), and hypotension in experimental animals. TNFα or IL-1 doses, which individually would produce no significant hemodynamic disarray, cause hypotension or shock when infused together in animal models [4]. In addition, TNFα and IL-1 have a negative inotropic effect of the cardiac myocytes. The degree of myocyte shortening is further compromised in the presence of a higher level of TNFα and IL-1 [24]. These observations may explain why the myocardial function becomes so depressed in severe sepsis. Nevertheless, a hyperdynamic state with high cardiac output is the usual presentation in early stages of septic shock [25].

IL-6

IL-6 is produced by T cells, B cells, and endothelial cells. It induces T-cell and B-cell proliferation and the production of acute-phase proteins [26]; the function of these proteins is discussed later in the article.

IL-8

The role of IL-8 is to induce the production of interferon-γ, an important antiviral cytokine; it also acts as a chemotactic agent for neutrophils and T cells. It is secreted by activated macrophages, monocytes, and Kupffer cells [27].

HMGB-1

HMGB-1, the latest inflammatory cytokine identified in septic patients, is secreted by activated macrophages, but its level begins to rise relatively late (16 hours), as compared to TNFα (60 to 90 minutes) and IL-1 (180 minutes) after the initial stimulation by LPS and remains elevated in most patients with severe sepsis or septic shock. Nevertheless, contrary to TNFα, IL-1, IL-6, and IL-8, there is no correlation between the level of HMGB-1 and the severity of the infections [4,28,29]. The injection of HMGB-1 to murine models causes sepsis-like symptoms or even death [28].

MIF

MIF, a proinflammatory cytokine, promotes the expression of TNFα in response to infections. The production of MIF is further increased by the action of the newly formed TNFα, creating a reentry pathway [30]. The expression of TLR4 is also enhanced by MIF. The plasma level of MIF correlates with the severity of the infection [31]. The release of MIF occurs immediately following the activation of the macrophages and monocytes. Contrary to other cytokines, such as TNFα, IL-1, and IL-8, that require transcriptional nuclear activation, large amounts of MIF are stored in cytoplasmic vacuoles and are readily available for use [32].

NO

NO is a free radical with cytotoxic properties. Its formation is induced by 3 isoforms of NO synthases (NOS): (1) endothelial cell NOS, (2) brain NOS, and (3) inducible macrophage-derived NOS. Under normal physiological conditions, only the endothelial and brain NOS are detectable. The inducible-NOS production can be triggered by either endotoxin, TNFα, IL-1, or PAF. The biological action of NO is mediated through the cyclic guanosine monophosphate system, which reduces the intracellular calcium concentration. This results in the relaxation of vascular and nonvascular smooth muscles, contributing to the hypotensive state in sepsis. NO also causes myocardial depression in endotoxin-induced shock in guinea pigs. In addition, NO inhibits platelet adhesion/aggregation and polymorphonuclear chemotaxis [33,34].

Complements

Endotoxin is the principal factor causing the activation of the complements via the alternate pathway. The activation of C3 by LPS is the initial and most important signaling reaction in the complement cascade in sepsis. Activated C3 and C5 (C3a, C5a) are potent vasodilators. They cause the release of histamine and act with kinin to enhance capillary permeability. C5a, a potent chemotactic factor for polymorphonuclears, facilitates phagocytosis of bacteria, host cell injury/death, or even organ failure. Excessive activation of complement components is detrimental to the host organs and may

Table 2. Partial List of Major Acute-Phase Proteins in Sepsis

Acute-Phase Protein	Actions
C-reactive protein	Facilitation of phagocytosis, opsonization of bacteria
Serum amyloid	Prevention of initiation of nuclear antigen–specific autoimmunity
Protease inhibitors	Neutralization of neutrophil proteases, ? protection against development of acute lung injury
Coagulation proteins (fibrinogen, plasminogen activator inhibitor, von Willebrand factor, heparin co-factor II)	Activation of coagulation, reduced thrombomodulin level, decreased protein Ca
α1-acid glycoprotein	? Protection against thermal injury
Haptoglobulin, ferritin	? Inhibition of microorganism replication by reduction of iron storage

contribute to the hemodynamic collapse in septic shock [35].

Eicosanoids

The systemic inflammatory response is capable of liberating PAF and arachidonic acid from the cell membrane. The key enzyme for the metabolic degradation of the membrane phospholipids is phospholipase A2 (PLA2), which is secreted in 3 isoforms: 2 secretary forms and a cytosolic form. The secretary forms are of 2 types: PLA2-I, secreted by the pancreas and involved in the digestive system, and PLA2-II, released by inflammatory cells, neutrophils, and mast cells. LPS, TNFα, and IL-1 can all induce the release of PLA2-II, in which the serum level varies proportionally with the severity of shock in septic patients. The third isoform is the cytosolic PLA2-III, a calcium-dependent enzyme that mediates intracellular signaling probably through protein kinase and GTP [36]. Eicosanoids designate the families of prostaglandins and leukotrienes because of their particular molecular structure that is made of 20-carbon essential fatty acids with variable double bonds.

Prostaglandins. The first step in the synthesis of prostaglandins from arachidonic acid is facilitated by the microsomal enzyme cyclo-oxygenase. The major vasoactive metabolites are PGE2, PGI1 (prostacyclin), and thromboxane A2 (TXA2). PGE2 and PGI2 cause prominent hypotension; PGI2 has 5 times more vasodilating potency than PGE2. In addition, PGI2 possesses anti-inflammatory properties by downregulating TNFα release in sepsis [37]. Conversely, TXA2 is a potent vasoconstrictor; it plays an important role in the development of pulmonary airway resistance, decreased compliance, and hypoxemia. PGD2 may cause a dose-dependent systemic vasoconstriction or vasodilation, but its action on pulmonary circulation is only vasoconstrictive. PGF2 does not alter blood pressure in humans [38]. The net effects of prostaglandins in sepsis are not uniform and may have conflictive physiologic properties.

Leukotrienes. Leukotrienes are formed by the action of lipoxygenase on arachidonic acid. The ensuing metabolites, LTC4, LTD4, and LTE4 (also called slow-reacting substances of anaphylaxis) may contribute to pulmonary capillary permeability and bronchospasm. Although they may be involved in vascular tone regulation, they do not seem to play an important role in the hypotensive state in sepsis [35]. The unique role of LTB4 is chemotactic for polymorphonuclears, leukocytes, eosinophils, and monocytes; it does not cause vasoconstriction or bronchoconstriction like its homologues [38].

PAF

PAF is synthesized by endothelial cells, macrophages, and neutrophils. It is released from membrane phospholipids by phospholipase A2 in response to endotoxin and promotes the aggregation/activation of platelets, thrombosis, and vascular injury. It mediates the release of histamine and serotonin from platelets; it also enhances the up-regulation of adhesion molecules and the chemotactic response of polymorphonuclear cells. The activated endothelial cells interact with circulating neutrophils to cause tissue injury and ischemia [39,40]. This paradigm is proposed as one of several hypotheses on the pathogenesis of multiple organ dysfunction in sepsis.

Acute-Phase Response

The acute-phase response is characterized by the secretion of a series of proteins, called acute-phase proteins. They are produced predominantly by the liver in response to inflammation or tissue injury, but their exact role is not completely understood [41]. IL-6 is the main inducer of the acute-phase proteins. The list of the major acute-phase proteins is shown in Table 2.

C-reactive protein. C-reactive protein has binding ability to nuclear ribonucleoproteins, facilitating the clearance of nuclear materials of injured and necrotic tissues. Its opsonic properties may confer a protective effect against infection. C-reactive protein is also capable of activating IL-6, causing a revolving effect on acute-phase protein production [41,42].

Serum amyloid. Serum amyloid derives from apolipoproteins, found in high-density lipoproteins. It may alter the metabolism of cholesterol, but its main biological action is believed to prevent the initiation of nuclear antigen–specific autoimmunity [42].

Protease inhibitors. Protease inhibitors (α1-antitrypsin, α1-antichymotrypsin) neutralize the neutrophil proteases. The up-regulation of these protease inhibitors may have a protective function against the development of acute lung injury [41].

Coagulation proteins. Coagulation proteins (plasminogen activator inhibitor [PAI], fibrinogen, von Willebrand factor, heparin cofactor II) are produced in larger quantities in response to the activation of the coagulation system. IL-6 suppresses the production of thrombomodulin, on which the anticoagulant factor protein C depends for its activation. The thrombomodulin level is further depressed by the constant binding to newly formed thrombin. Activated protein C (protein Ca) exerts anti-inflammatory properties. Administration of protein Ca in septic patients resulted in dose-dependent reductions of TNFα, IL-6, coagulation markers, and endothelial cell expression of adhesive molecules [43]. Protein Ca increases the expression of the following antiapoptotic genes: cell cycle–related human Gu hilicase, A1 BcI-2, and IAP homologs. Furthermore, the expression of the proapoptotic genes, TRMP-2 and calreticulin, is also suppressed by protein Ca [44]. The depletion of protein Ca in septic patients further impairs their ability to maintain coagulation homeostasis and to muffle the systemic inflammatory response. Mortality in sepsis is inversely proportional to the plasma level of protein Ca [45].

Albumin. Albumin's plasma concentration drops in response to IL-6. This may result in low osmotic pressure and significant reduction in the pharmacokinetic property of albumin-binding drugs [41].

Other proteins. The level of α1-acid glycoprotein, haptoglobin, ceruloplasmin, ferritin, and hemopexin is enhanced in sepsis, but their roles are only speculative [41].

Endogenous Anti-inflammatory Response

The systemic inflammatory responses to sepsis lead to physiologic alterations with catastrophic consequences if left unchallenged. The down-regulation of the inflammatory mediators by endogenous substances abrogates the excessive and overwhelming inflammatory response (Table 1). The biology of these anti-inflammatory substances is reviewed below.

IL-10

IL-10 is an anti-inflammatory cytokine with down-regulatory effects on macrophages. It is synthesized by epithelial cells, monocytes, and lymphocytes in response to inflammatory conditions. Its level correlates with the state of shock, TNFα plasma concentration, and organ injuries [46]. It is shown to decrease mortality in experimental septic animals [47,48].

Soluble TNFα Receptors

During the course of sepsis, the extracellular domains of TNFα receptors shed and circulate freely in the plasma; nevertheless, they still maintain their binding ability and affinity with TNFα. These naturally formed blocking receptors are called "soluble receptors to TNF" or "TNF-binding proteins" [4]. Mortality is shown to decrease significantly in septic-induced murine models after infusion of TNF-binding protein immunoglobulin [49]. Conversely, the administration of etanercept, a dimeric fusion of human TNF-binding protein and the Fc portion of IgG1, has been associated with serious infections or even sepsis in recipients treated for rheumatoid arthritis [50].

IL-1 Receptor Antagonists

IL-1 receptor antagonists (IL-1-Ra) are naturally produced by monocytic leukocytes following IL-1 release. They block the biologic activity of IL-1 by competitively inhibiting IL-1 binding to an IL-1 receptor. Unlike TNFα receptors, shedding of IL-1 does not occur naturally [4]. IL-1-Ra has been genetically engineered (anakinra) and commercially used in the treatment of rheumatoid arthritis; the risk of infection also increases with its administration [50].

Prostaglandin I2

Synthesis of prostaglandins, as well as leukotrienes, is increased in sepsis. Abounded amount of PGI2 (prostacyclin) promotes the down-regulation of TNFα production. The physiologic effects of prostacyclin are not always beneficial in sepsis; its widespread vasodilating actions are contributory to the hypotensive state [37].

Heat Shock Proteins

Heat shock proteins are a specific group of intracellular proteins, which provide protective effects against thermal and sepsis-induced injuries. They are ubiquitous and inhibit TNFα and IL-1 release. They also increase the expression of the IkBα gene, which has negative feedback to NF-κB activation [51].

Phosphatases

The mechanism by which the nuclear factor is activated is by the phosphorylation of signaling proteins mediated by kinases. That process can be reversed by phosphatases, which act as dephosphorylators in the cytoplasma. They are categorized into 3 main classes based on the molecular residues targeted: (1) resine-threonine phosphatases, (2) tyrosine phosphatases, and (3) mixed kinase phosphatases. Leukocytes and endothelial cells are deactivated by their actions, causing a marked reduction of inflammatory mediators. Apoptosis is also down-regulated; this is attributed in part to the reduction of NF-κB–dependent production of TNFα [52]. The phosphatases play an important role in the regulation of the cellular transduction pathways.

Cortisol

The anti-inflammatory properties of cortisol are reputedly recognized and applied in clinical practice. Cortisol release is enhanced in response to stress, systemic inflammation, and sepsis. It inhibits the binding and transcription of NF-κB and prevents gene activation, resulting in a reduction of various NF-κB–dependent cytokines, such as TNFα, IL-1, and IL-6 [53]. The coupling of cortisol to the glucocorticoid receptor induces the liberation of heat shock protein, which is embedded in the glucocorticoid receptor in normal conditions [54]. Cortisol suppresses the activity of phospholipase A2 and inducible NOS, leading to a reduction of eicosanoids and NO, respectively [55].

The plasma cortisol level is usually normal or high in patients with severe sepsis and septic shock [56]. However, septic patients with adrenal insufficiency have a higher mortality [57].

Genetic Susceptibility

Predisposition for sepsis has been linked to the genomic fabric of susceptible individuals. The location of the TNFα gene in the short arm of chromosome 6 has raised speculation that the major histocompatibility complex (MHC), also located in the same region, exerts some influence on the production of TNFα. Striking similarities exist between the TNFα gene and the genes encoding human leukocyte antigen (HLA) class 1 and class 3 by the way these genes are expressed and organized. Individuals with HLA-DR2, HLA-DR5, and HLA-DQW1 exhibit low TNFα production (low responder phenotype), whereas the production of TNFα is higher in individuals with HLA-DR3 and HLA-DR4 (high responder phenotype) [22,58]. Patients with rheumatoid arthritis display high TNFα plasma level and express the HLA-DR4 in 60% to 70% of the cases versus only 25% to 30% of normal individuals [59].

Two biallelic TNF polymorphisms have been identified in the promoter region of the TNFα gene; they differ by the position of guanine or adenine at locus-308: TNF1 (guanine) and TNF2 (adenine), respectively. A greater concentration of serum TNFα and higher mortality were found in septic patients who carried the TNF2 allele as compared to individuals with the TNF1 type [60,61], but conflicting results were obtained in other studies, in which TNF2 was not found to be associated with increased mortality [62,63]. The TNF-β gene consists of 2 alleles: (1) TNF-β1, with guanine at position 1069 and susceptible to *Nco*I digestion, and (2) TNF-β2, with adenine at position 1069. Higher mortality and elevated TNFα plasma concentration were found in individuals with the TNF-β2 allele [64]; however, no correlation between TNFα production and TNF-β2 homozygotes was found in another study [65]. Furthermore, TNF-β1 homozygotes were found to release more TNFα than individuals with the TNF-β2 genotype in one study [66].

The IL-1-Ra gene contains several alleles characterized by the variable number of 86 base-pair tandem repeats. The IL-1-RaA2 allele (2 repeats) is

reported to be associated more frequently with patients with severe sepsis. The association of homozygous TNF-β2 and IL-1-RaA2 was shown to carry a mortality rate of 100% in septic patients [67].

The IL-10 genomic variation affects the expression of IL-10 in septic patients. The IL-10-592A allele expresses low IL-10 production and is associated with higher mortality [68]. Conversely, the IL-10-1082 G/G allele, a high IL-10 producer, was more common in patients with high severity scores and in septic patients with pneumonia [69]. Cross-analysis of these studies reveals differences in the applied methodologies, lack of diversity of the cohort samples, and inadequacy in the population sizes. The pursuit of a genomic map in the search of a predisposition factor for sepsis would help to identify high-risk genes and to segregate susceptible individuals for specific immunotherapy.

PAI-1 binds to fibrin and promotes the stability and extension of blood clots. PAI-1 gene expression can be activated by endotoxin, TNFα, or IL-1 [70]. Poor outcome in patients with sepsis and septic shock was found to correlate with a high plasma concentration of PAI-1 [71]. Polymorphisms in the PAI-1 gene are ascribed to an aberration to a common single-base pair, in which the depletion of a guanine residue gives rise to 4G and 5G alleles [72]. In one study of children with meningococcal sepsis, the 4G/4G genotype group had a higher plasma level of PAI-1, worse score in illness severity, and greater relative risk of death than those with 4G/5G and 5G/5G genotypes [73]. Even trauma patients with the 4G allele displayed a higher prevalence of sepsis and multiple organ failure. In this study, mortality among the 4G/4G genotype was 58%, as compared to 28% for 4G/5G and 15% for 5G/5G genotypes [74].

Multiple Organ Dysfunction

Multiple organ dysfunction or failure (MOF) is the culmination of tissue injuries in severe sepsis. Mortality increases as the number of organs failing increases [75]. Although microorganisms and theirs toxins may inflict substantial tissue damages, it is believed that MOF is mainly caused by the host's own endogenously produced mediators. Compelling evidence suggests that inflammatory mediators contribute to end-organ failure. Inflammatory mediators, such as TNFα, IL-1, PAF, NO, and HMGB-1, are shown to cause a state of shock when infused individually to experimental animals. The compiling actions of these mediators optimize the hypotensive state and circulatory collapse. TNFα induces host cell injury and apoptosis in a disproportionately high rate because of its excessive release in severe sepsis. TNFα level is higher among nonsurvivors than survivors in septic shock [4].

Activated macrophages and neutrophils trigger the release of adhesion molecules from the endothelial cells. PAF, a potent chemotactic factor for neutrophils, contributes significantly to the initiation of the leukocyte adhesion, coagulation cascade, and microvascular occlusion, which eventually lead to tissue ischemia. Inadequate tissue perfusion and hypoxia favor the conversion of xanthine dehydrogenase to xanthine oxidase, which catalyzes the transformation of oxygen to free radicals, such as superoxide, hydrogen peroxide, and hydroxyl radical. These oxygen free radicals further amplify tissue injuries and organ failure. Resuscitative therapy aimed at the restoration of tissue perfusion facilitates the propagation of free radicals systemically, well beyond the ischemic territory. In the setting of reperfusion, vascular permeability increases, and the vascular tone loses its autoregulation in response to the effects of inflammatory mediators. Reperfusion injury may cause more harm than the ischemia itself [76].

The source of the infection is not identified in 30% of patients with bacteremia and MOF [77]. It is speculated that bacterial dislocation from the gut of critically ill patients is the port of entry for bacteria; indeed, many of these bacteria are normally found in the enteric flora [78]. Increasing vascular permeability and/or ischemic-related injury to the intestinal wall are believed to promote the bacterial translocation to the bloodstream.

Although the pathophysiology of MOF is only speculative, the current consensus points to the inflammatory response by mediators, ischemic-reperfusion injury, oxygen free radicals, and gut-related bacteremia, acting alone or, most likely, in concert to cause end-organ failure.

Conclusion

Factors that control the modulation of the immune responses originate not only from the infective organisms but also from the released inflammatory mediators, which act in a paracrine and autocrine manner. TNFα and IL-1 are the principal and initial cell-mediated cytokines relegated to the host defense against infection. While cytokines are essential to the recruitment of inflammatory factors (cellular and humoral), their excessive production brings about irreversible damage to the host tissues. In septic shock, the mortality is consequential to refractory hypotension and MOF. Detrimental effects of endotoxin, cytokines, and noncytokine mediators play an important role

in the development of circulatory collapse and cell death. These observations suggest that the pathogenesis of multiple organ failure is multifactorial.

Inborn responses from the host immune cells also produce anti-inflammatory mediators, which mitigate the inflammatory response. These naturally produced substances deactivate the monocytic lymphocytes, endothelial cells, and NF-κB, causing de facto the down-regulation of TNFα and IL-1. The autoregulatory balance between the inducive and suppressive mediators is paramount to prevent overwhelming immune responses and to reduce host cell demise and apoptosis. Pharmacologic manipulation of the anti-inflammatory mediators and the blockage of the major cytokines may hold the key to a more successful treatment of severe sepsis in the future; their potential use as therapeutic tools has been vigorously explored in contemporary clinical research.

References

1. Linde-Zwirble WT, Angus DC, Carcillo J, Lidicker J, Clermont G, Pinsky MR. Age-specific incidence and outcome of sepsis in the US: analysis of incidence, outcome, and associated cost of care. *Crit Care Med*. 1999;27(suppl 1):A33.
2. Albert C, Brun-Buisson C, Burchardi H, et al. Epidemiology of sepsis and infection in ICU patients from an international multicenter cohort study. *Intensive Care Med*. 2002;28:108-121.
3. Sands KE, Bates DW, Lanken PN, et al. Epidemiology of sepsis syndrome in 8 academic medical centers. Academic Medical Center Consortium Sepsis Project Working Group. *JAMA*. 1997;278:234-240.
4. Shapiro L, Gelfand JA. Cytokines and sepsis: pathology and therapy. *New Horiz*. 1993;1:13-22.
5. Corriveau CC, Danner RL. Endotoxin as a therapeutic target in septic shock. *Infect Agents Dis*. 1993;2:35-40.
6. Takeuchi O, Hoshino K, Kawai T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacteria cell wall components. *Immunity*. 1999;11:443-451.
7. Peters CJ, Zaki S. Role of the endothelium in viral hemorrhagic fevers. *Crit Care Med*. 2002;30(suppl):S268-S273.
8. Crislip MA, Edwards JE Jr. Candidiasis. *Infect Dis Clin North Am*. 1989;3:103-110.
9. Manthous CA, Hall JB, Samsel RW. Endotoxin in human disease. Part 1: biochemistry, assay, and possible role in diverse states. *Chest*. 1993;104:1572-1581.
10. Young LS, Gascon R, Alam S, et al. Monoclonal antibodies for treatment of gram-negative infections. *Rev Infect Dis*. 1989;11(suppl 7):S1564-S1571.
11. Teng NN, Kaplan HS, Herbert JM, et al. Protection against gram-negative bacteremia and endotoxemia with human monoclonal IgM antibodies. *Proc Natl Acad Sci U S A*. 1985;82:1790-1794.
12. Wang H, Vishnubhakat JM, Bloom O, et al. Proinflammatory cytokines (tumor necrosis factor and interleukin 1β) stimulate release of high mobility group protein-1 by pituicytes. *Surgery*. 1999;126:389-392.
13. Wright SD. CD14 and innate recognition of bacteria. *J Immumol*. 1995;155:6-8.
14. Lien E, Ingalls RR. Toll-like receptors. *Crit Care Med*. 2002;30(suppl 1):S1-S11.
15. Visintin A, Mazzoni A, Spitzer JA, et al. Secreted MD-2 is a large polymeric protein that efficiently confers lipopolysaccharide sensitivity to toll-like receptor 4. *Proc Natl Acad Sci U S A*. 2001;98:12156-12161.
16. Arbabi S, Maier RV. Mitogen-activated protein kinases. *Crit Care Med*. 2002;30(suppl 1):S74-S79.
17. Tanowitz HB, Chan J. Gram-positive sepsis. *Crit Care Med*. 2000;28:3081-3082.
18. Sriskandandan S, Cohen J. Gram-positive sepsis mechanisms and differences from gram-negative sepsis. *Infect Dis Clin North Am*. 1999;13:392-412.
19. Tyler UL, Fields BN. Introduction to virus and viral diseases: In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 4th ed. New York, NY: John Wiley & Sons; 1995.
20. Hadley S, Lee WW, Ruthazer R, Nasraway SA. Candidemia as a cause of sepsis shock and multiple organ failure in immunocompromised patients. *Crit Care Med*. 2002;30:1808-1814.
21. Williams G, Giroir BP. Regulation of cytokine gene expression: tumor necrosis factor, interleukin-1, and emerging biology of cytokine receptors. *New Horiz*. 1995;3:276-287.
22. Murphy K, Haudek SB, Thompson M, Giroir BP. Molecular biology of septic shock. *New Horiz*. 1998;6:181-193.
23. Mahidara R, Billiar TR. Apoptosis in sepsis. *Crit Care Med*. 2000;28(suppl):105-113.
24. Kumar A, Thota V, Dee L, et al. Tumor necrosis factor-α and interleukin-1β are responsible for in vitro myocardial cell depression induced by human septic shock serum. *J Exp Med*. 1996;183:949-958.
25. Parker MM, Shelhamer JH, Natanson C, et al. Serial cardiovascular variables in survivors and non-survivors of human septic shock: heart rate as an early predictor of prognosis. *Crit Care Med*. 1987;15: 923-929.
26. Gauldie J, Richards C, Harnish D, et al. Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci U S A*. 1987;84: 7251-7255.
27. Oberholzer A, Oberholzer C, Moldaver LL. Cytokine signaling-regulation of the immune response in normal and critically ill states. *Crit Care Med*. 2000;28(suppl):N3-N12.
28. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science*. 1999;285:248-251.
29. Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB-1) in patients with severe sepsis and septic shock. *Crit Care Med*. 2005;33:564-573.
30. Calandra T, Bernhagen J, Mitchell RA, et al. The macrophage is an important and previously unrecognized source of macrophage migratory inhibitory factor. *J Exp Med*. 1994;179: 1895-1992.
31. Calandra T, Echtenacher B, Le Roy D, et al. Protection from septic shock by neutralization of macrophage migratory inhibitory factor. *Nat Med*. 2000;6:164-169.
32. Leng L, Bucala R. Macrophage migration inhibitory factor. *Crit Care Med*. 2005;33(suppl):S475-S477.
33. Szabo C. Alterations in nitric oxide production in various forms of circulatory shock. *New Horiz*. 1995;3:1-32.
34. Brady AJB, Pode-Wilson PA, Harding SE, et al. Nitric oxide production with cardiac myocytes reduces their contractility in endoxemia. *Am J Physiol*. 1992;263:H1963-H1966.
35. Dries DJ. Activation of the clotting system and complement after trauma. *New Horiz*. 1996;4:276-288.
36. Bulger EM, Maier RV. Lipid mediators in the pathophysiology of critical illness. *Crit Care Med*. 2000;28(suppl):N27-N36.
37. Oettinger W, Berger D, Berger HG. The clinical significance of prostaglandins and thromboxane as mediators of septic shock. *Klin Wochenschr*. 1987;65:61-68.
38. Campbell WB, Halushka PV. Lipid-derived autocoids. In: Hardman JG, Limbird LE, eds. *Goodman & Gilman's Pharmacological Basis of Therapeutics*. 9th ed. New York, NY: McGraw-Hill; 1996: 601-616.
39. Zimmernan GA, Elstad MR, Lorant DE, et al. Platelet-activating factor: new roles in cell-cell interactions. *J Lipid Mediat Cell Signal*. 1994;10:S7-S59.

40. Ayala A, Chaudry IH. Platelet activating factor and its role in trauma, shock, and sepsis. *New Horiz*. 1996;4:265-275.
41. Pannen BHJ, Robothan JL. The acute-phase response. *New Horiz*. 1995;3:183-197.
42. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component, and serum amyloid A protein. *Immunol Today*. 1994;15:81-88.
43. Hartman DL, Bernard GR, Helterbrand JD, Fisher CJ. Recombinant human activated protein C (rhAPC) improves coagulation abnormalities associated with severe sepsis [abstract]. *Intensive Care Med*. 1998;24(suppl 1):S77.
44. Joyce DE, Gelbert L, Ciaccia A, et al. Gene expression profile of antithrombic protein C defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem*. 2001;276: 11199-11203.
45. Fisher CJ, Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. *Crit Care Med*. 2000;28: S49-S56.
46. Scumpia PO, Moldawer LL. Biology of interleukin-10 and its regulatory roles in sepsis. *Crit Care Med*. 2005;33(suppl): S468-S471.
47. Oberholzer C, Oberholzer A, Bahjat FR, et al. Targeted adenovirus-induced expression of IL-10 decreases thymic apoptosis and improves survival in murine sepsis. *Proc Natl Acad Sci U S A*. 2001;98:11503-11508.
48. Oberholzer A, Oberholzer C, Bahjat FR, et al. Increased survival in sepsis by in vivo adenovirus-induced expression of IL-10 in dentritic cells. *J Immunol*. 2002;168:3412-3418.
49. van Zee KS, Kohno T, Fischer E, et al. Tumor necrosis soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor in vitro and in vivo. *Proc Natl Acad Sci U S A*. 1992;89:4845-4849.
50. Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. *N Engl J Med*. 2004;350:2167-2179.
51. Christians ES, Yan LJ, Benjamin IJ. Heat shock factor 1 and heat shock proteins: critical partners in protection against acute cell injury. *Crit Care Med*. 2002;30(suppl):S43-S50.
52. Shanley TP. Phosphatases: counterregulatory role in inflammatory cell signaling. *Crit Care Med*. 2002;30(suppl):S80-S88.
53. Ray A, Prefontaine KE. Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. *Proc Natl Acad Sci U S A*. 1994;91:752-756.
54. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem*. 1994;63:451-486.
55. Radonski MW, Balmer RM, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci U S A*. 1990;87:10043-10047.
56. Fourrier F, Bricard H, Cohen Y. Corticotherapie au cours du choc septique et du syndrome de detresse respiratoire de l'adulte. *Réanim Urg*. 2000;9:597-603.
57. Annane D, Sebille V, Troche G, et al. A 3-level prognostic classification in septic shock based on prognostic levels and cortisol response to corticotropin. *JAMA*. 2000;283:1038-1045.
58. Brinkman BM, Huizinga TWJ, Kurban SS, et al. Tumor necrosis factor-α gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? *Br J Rheumatol*. 1997;36:516-521.
59. Arnett FC. Rheumatoid arthritis. In: Goldman L, Bennett JC, eds. *Cecil Textbook of Medicine*. 21st ed. Philadelphia, Pa: W. B. Saunders Co; 2000:1492-1499.
60. Mira JP, Cariou A, Grall F, et al. Association TNF2, a TNF-α promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA*. 1999;282:561-568.
61. Appoloni O, Dupont E, Vandercruys M, et al. Association of tumor necrosis factor 2 allele with plasma tumor necrosis factor alpha levels and mortality from sepsis shock. *Am J Med*. 2001;110:486-488.
62 Waterer GW, Quasney MW, Cantor RM, et al. Septic shock and respiratory failure in community-acquired pneumonia have different TNF polymorphism associations. *Am J Respir Crit Care Med*. 2001;163:1599-1604.
63. Reid CL, Perrey C, Pravica V, et al. Genetic variation in pro-inflammatory and anti-inflammatory cytokine production in multiple organ dysfunction syndrome. *Crit Care Med*. 2002;30: 2216-2221.
64. Pociot F, Briant L, Jongenneel CV, et al. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles wit the secretion of TNFα and TNFβ by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol*. 1993;23:224-231.
65. Stuber F, Udalova IA, Book M, et al. –308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to liposaccharide inducibility of the human TNF promoter. *J Inflamm*. 1996; 46:42-50.
66. Derkx BHF, Bruin KF, Jongenneel CV, et al. Familial differences in endotoxin-induced TNF release in whole blood and peripheral blood mononuclear cells in vitro; relationship to HLA-haptotype and TNF gene polymorphism. In: Bruin KF, ed. *Endotoxin Responsiveness in Humans*. Amsterdam, the Netherlands: Thesis; 1994:51-64.
67. Fang XM, Schroder S, Hoeft A, et al. Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis. *Crit Care Med*. 1999;27: 1330-1334.
68. Lowe PR, Galley HF, Abdel-Fattah A, et al. Influence of interleukin-10 expression and survival in critically ill patients. *Crit Care Med*. 2003;31:34-38.
69. Gallagher PM, Lowe G, Fitzgerald T, et al. Association of IL-10 polymorphism with severity of illness in community acquired pneumonia. *Thorax*. 2003;58:154-156.
70. Texereau J, Pene F, Chiche JD, et al. Importance of hemostatic gene polymorphisms for susceptible to and outcome of severe sepsis. *Crit Care Med*. 2004;32(suppl):S313-S319.
71. Mesters RM, Florke N, Ostermann H, et al. Increase of plasminogen activator inhibitor levels predicts outcome of patients with sepsis. *Thromb Haemost*. 1996;75:902-907.
72. Westendorp RG, Hottenga JJ, Slaboom PE. Variation in plasminogen activator-inhibitor-1 gene and risk of meningococcal septic shock. *Lancet*. 1999;354:561-563.
73. Hermans PW, Hibberd ML, Booy R, et al. 4G/5G promotor polymorphism in the plasminogen activator-inhibitor 1 gene and outcome of meningococcal disease. Meningococcal Research Group. *Lancet*. 1999;354:555-560.
74. Menges T, Hermans PW, Little SG, et al. Plasminogen activator-inhibitor-1 4G/5G promoter polymorphism and prognosis of severely injured patients. *Lancet*. 2001;357: 1096-1097.
75. Ruokonen E, Takala J, Kari A, Alhava E. Septic shock and multiple organ failure. *Crit Care Med*. 1991;19:1146-1151.
76. Livingston DH, Mosenthal AC, Deitch EA. Sepsis and multiple organ dysfunction syndrome: a clinical-mechanistic overview. *New Horiz*. 1995;3:257-266.
77. Goris RJA, te Boekhorst TPA, Nuytinck JKS, et al. Multiple organ failure: generalized autodestructive inflammatory. *Arch Surg*. 1985;120:1109-1115.
78. Garrison RN, Fry DE, Berborich S, et al. Enterococcal bacteremia: clinical implications and determinants of death. *Ann Surg*. 1982;196:43-47.