

The Pathophysiology of Septic Shock

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KEYWORDS

- Sepsis • Severe sepsis • Septic shock
- Myocardial depression • Vasodilation

The word “sepsis” comes from the Greek word *sepo* meaning decay or putrefaction, and its original usage described the decomposition of organic matter in a manner that resulted in decay and death.¹ In the Hippocratic model of health and disease, living tissues broke down by 1 of 2 processes. Pepsis was the process through which food was digested, leading to health. Sepsis, however, denoted tissue breakdown that resulted in disease. Hippocrates used this term to describe the process of abnormal tissue breakdown that resulted in a foul odor, pus-formation, and sometimes dead tissue.² This usage of the term sepsis persisted for almost 3 millennia, and subsequent work establishing a causal link between microbes and suppurative infections, or systemic symptoms from infection, did not change the use of the term as a description of a constellation of clinical findings, but rather established infection as the underlying cause.³ The term “shock” comes from the French word *choquer* meaning “to collide with,” and aptly describes the body’s response to invading microbes and, to a large extent, its disruptive effect on normal physiology. Initially used in the medical literature in the 1700s, its earliest uses connoted a sudden jolt that often led to death (the initial physical injury). This definition evolved to describe widespread circulatory dysfunction following injury.⁴

Sepsis is the systemic maladaptive response of the body to the invasion of normally sterile tissue by pathogenic, or potentially pathogenic, microorganisms. Shock may be defined as a “state in which profound and widespread reduction of effective tissue perfusion leads first to reversible, and then, if prolonged, to irreversible cellular injury.”⁵ From a clinical standpoint, this progressive cellular dysfunction manifests as a continuum from sepsis, to severe sepsis, and finally to septic shock (**Box 1**).

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Box 1**Definitions of sepsis, severe sepsis, and septic shock**

Sepsis: sepsis is defined as infection plus systemic manifestations of infection

Severe sepsis: sepsis with sepsis-induced organ dysfunction or tissue hypoperfusion

Sepsis-induced hypotension: a systolic blood pressure (SBP) less than 90 mm Hg or mean arterial pressure less than 70 mm Hg, or an SBP decrease of greater than 40 mm Hg or greater than 2 SD less than normal for age in the absence of other causes of hypotension

Septic shock: sepsis-induced hypotension persisting despite adequate fluid resuscitation.

Sepsis-induced tissue hypoperfusion: septic shock, lactate elevation beyond the upper limits of normal or oliguria

Acute oliguria: urine output less than 0.5 mL/kg/h for at least 2 hours, despite adequate fluid resuscitation

Data from Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008;36:296–27.

Severe sepsis and septic shock are the end result of complex interactions between infecting organisms and several elements of the host response, and reflect a primarily inappropriate response by the host to a microbial pathogenic insult. The key term that describes the pathophysiologic events in septic shock at any point in time is the “mismatch” of the host response to the intensity of the pathogenic stimuli ultimately leading to organ injury or dysfunction with or without hypotension. This mismatch results in, amongst other derangements, an immune profile that could be predominantly proinflammatory (systemic inflammatory response syndrome [SIRS]), mixed (mixed antagonistic response syndrome [MARS]), or predominantly anti-inflammatory (compensatory anti-inflammatory syndrome [CARS]). The nature of the interactions between the microbial pathogen and the host is complex and, at the tissues, results in excessive inflammation or immunosuppression, abnormal coagulation and blood flow, and microcirculatory dysfunction leading to organ injury and cell death (**Fig. 1**).

The complex events that occur in septic shock can be broadly divided into micro-organism-related components and host-related components. The broad categories are further subdivided into cellular and humoral components. Pathogen-related events in the pathophysiology of septic shock include the mechanisms by which microbes evade host defenses and subvert aspects of the host immune response, resulting in significantly increased morbidity. Concerning the host-related events in septic shock, multiple derangements involving several biologic systems contribute to different degrees to the development of septic shock. A meaningful review of every proven or proposed pathogenetic mechanism for septic shock is near impossible, and this article focuses on selected dysfunctions believed to play more significant roles in the development of septic shock. These are outlined below, and include aspects of microbial pathogenicity, key cellular and humoral aspects of the maladaptive immunoinflammatory response, the interactions between the immunoinflammatory and coagulation systems, and their cardiocirculatory consequences, resulting in the clinical picture of septic shock.

- The role of the pathogen
- Immunoinflammatory dysfunction leading to severe sepsis
 - Pathogen recognition
 - Pro- and anti-inflammatory cellular signaling/signal transduction

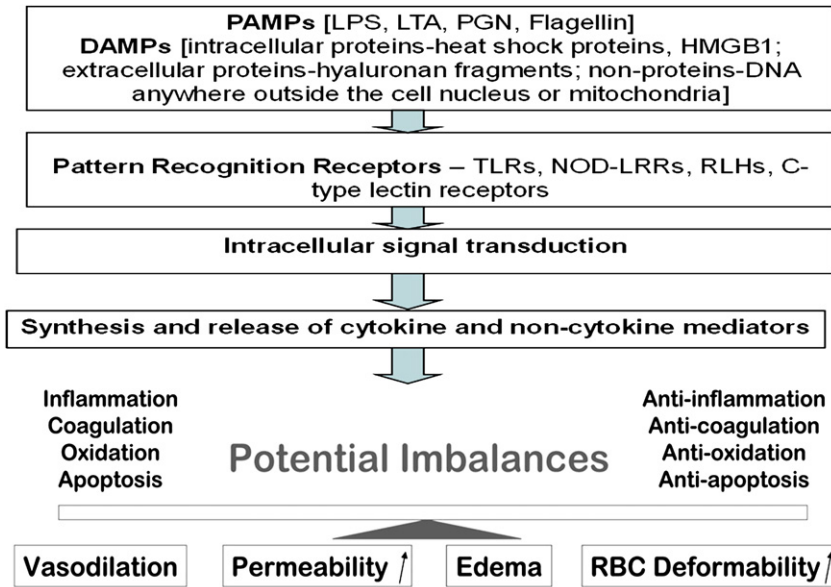


Fig. 1. Pathophysiology of severe sepsis and septic shock. (Adapted from Cinel I, Opal SM. Molecular biology of inflammation and sepsis: a primer. Crit Care Med 2009;37(1):293; with permission.)

- Release of pro-and anti-inflammatory mediators
 - Immune and non-immune effector cell dysfunction
 - Interactions with other biologic systems in sepsis
- Cardiocirculatory dysfunction in severe sepsis resulting in progression to septic shock

THE ROLE OF THE PATHOGEN

The initial event in severe sepsis and septic shock involves the invasion of normally sterile tissue by pathogenic microbes. The interactions between the pathogens and the host immune system may result in either a contained infectious process with minimal tissue injury or severe sepsis and septic shock. The abnormal host response seen in septic shock can be triggered by bacteria, viruses, or fungi.

Historically, our understanding of the pathophysiologic events in sepsis has focused on the maladaptive responses by the host, minimizing the role, if any, played by the pathogen invaders. Emerging evidence suggests a more significant role for pathogens than was previously believed.⁶ The development of modern imaging techniques with differential spatial and temporal resolution has provided the means to study the complex interactions between pathogens and their mammalian hosts, leading to advances in our understanding of bacterial pathogenicity. It is known that bacterial and nonbacterial pathogens possess an array of specific mechanisms (virulence factors) that confer the ability to evade host defense mechanisms and proliferate in host tissues (Box 2, Fig. 2). Bacterial virulence factors are better studied than their nonbacterial counterparts, and these mechanisms vary across species, classes, and strains of bacteria. Despite wide variation in the nature of the pathogens and their virulence factors, some common themes have emerged with regard to how pathogens subvert the early immune response and exert their full pathogenic potential.

Box 2**Virulence characteristics of bacterial pathogens***Mechanisms of bacterial adherence to host epithelial surfaces*

Adhesins: bacterial protein products (integral or secreted) that enable pathogenic organisms to bind onto host tissue elements (eg, collagen fibers)

Flagella, fimbriae, and pili: bacterial appendages whose primary role is mobility, but they enable pathogens to directly attach themselves to host cells and extracellular matrix components

Type III secretion system: functions as a molecular conduit, enabling bacteria to introduce proteins into host cells, altering their function to enhance bacterial survival

Ligand mimicry: by producing proteins similar to host-derived proteins, bacteria are able to bind to relevant ligand receptors

Mechanisms of bacterial invasion following adherence

Bacterial protein secretion systems I, II, III, IV: specialized transporter systems that enable the delivery of bacterial products into the extracellular matrix (I and II) or intracellularly (III and IV), facilitating tissue invasion and intracellular infection

Lipidrafts: avoiding the apical areas of epithelial cells exposed to commensals and resistant to pathogen invasion, pathogenic bacteria bind to the baso-lateral aspects of the host cell plasma membrane. This area is rich in cholesterol and pathogen recognition receptors. Following binding to plasma membrane cholesterol, the pathogens form intracellular vacuoles, rendering them immune to lysosomal endocytosis.

Bacterial host defense evasion mechanisms

Anti-phagocytosis: bacterial pathogens have several mechanisms to avoid phagocytosis: (1) inhibition of opsonization by encapsulation; (2) surface antigenic variation that prevents recognition as pathogens; (3) inhibition of uptake via the release of toxic protein effectors; (4) intracellular survival and replication in the cytosol and also within lysosomes; (5) induction of immune effector cell apoptosis.

Biofilm formation: a biofilm is a polysaccharide matrix that encapsulates entire bacterial colonies. In addition to being protected from phagocytosis, these bacterial colonies are largely immune to antibiotic drug action by existing in a dormant state. Dead tissue and foreign bodies provide optimal conditions for biofilm formation.

Virulence factor-mediated host immune dysfunction

Discussed in section on immunoinflammatory dysfunction

Virulence factor-induced host tissue injury

Discussed in sections on immunoinflammatory dysfunction and host organ/cellular injury/dysfunction in septic shock

Quorum-sensing, Cell-to-cell Signaling and Coordinated Gene Expression

Following pathogen adherence to an epithelial surface, specific mucosal defense mechanisms are triggered by the host to suppress pathogen proliferation and prevent invasion of the epithelial barrier. These include secretion of a mucus layer, epithelial cell shedding, and secretion of enzymes such as lysozyme. To establish infection, bacteria must be able not only to evade these additional host defense mechanisms but also to produce virulence factors to facilitate invasion. Expression of virulence factors by a single bacterium is highly unlikely to lead to established infection, much less tissue damage. Therefore the bacterial inoculum or population density to some extent affects the development and severity of infection. The critical bacterial density needed to initiate an infectious process is referred to as a quorum. Bacteria have developed systems of cell-to-cell communication that enable them to assess

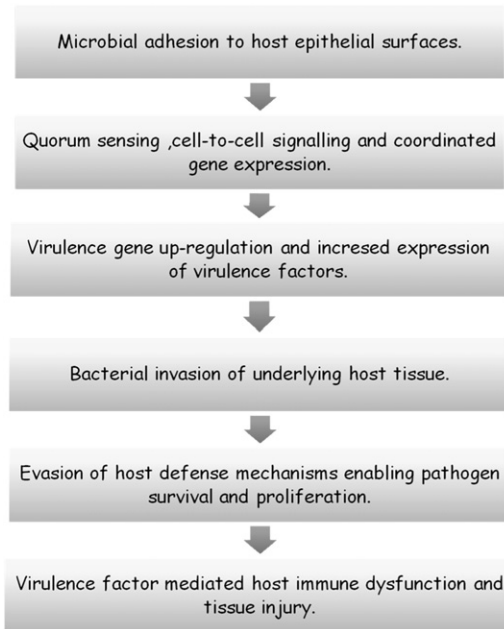


Fig. 2. Sequence of events leading to established infection in human hosts.

their population density and react to their environment as a population, increasing their chances of overwhelming host defense mechanisms and establishing infection.

These bacterial cell-to-cell signaling systems are called quorum-sensing systems (QSSs), and result in coordinated gene activation and expression of high concentrations of extracellular virulence factors by the entire bacterial population. QSSs are described in Gram-positive and Gram-negative bacteria involved in human sepsis, and involve the secretion of signaling molecules called autoinducers, with the autoinducer concentration tightly linked to the regulation of key aspects of genetic expression.^{7,8}

Quorum sensing allows both intra- and interspecies bacterial cell-to-cell communication. Animal experiments have demonstrated loss of microbial virulence with deletion of bacterial quorum-sensing genes and restoration of virulence following plasmid insertion. The ability to have virulence gene expression regulated by a global control system (ie, the QSS) prevents virulence factor expression or excessive proliferation when population densities are low, preventing premature pathogen detection. Thus QSSs play a major role in the regulation of biofilm synthesis.⁹ Once the critical population density is attained, virulence genes are expressed along with cellular proliferation signals, with swift tissue invasion and establishment of infection.

Recent experiments have shown that QSSs are capable of facilitating host-pathogen communication leading to pathogen-mediated modulation of host immune responses. Some QSSs can recognize and bind to human interferon- γ leading to subsequent expression of QSS genes.¹⁰ This suggests that the critical threshold for QSS gene expression may be somewhat host-dependent, with earlier activation if the host is sensed as being more susceptible.¹¹

There are 2 main bacterial QSSs. Gram-positive bacteria synthesize cytosolic autoinducers that are actively transported to the extracellular environment, where they bind to specific receptor proteins on neighboring bacteria, initiating a signaling cascade resulting in QSS control of relevant aspects of cellular function.¹²

Gram-negative autoinducers are termed acyl-homoserine lactones (AHL) and are produced by a different enzyme system (LuxI enzyme). After they are synthesized they diffuse passively between intra- and extracellular environments until critical population density (high signal molecule concentration) is achieved. At this point, the AHL proteins bind to the intracellular LuxR enzymes, forming a complex that acts on the promoter regions of QSS genes, leading to relevant gene expression.

Virulence Gene Upregulation and Increased Expression of Virulence Factors

QSS-regulated gene expression results in the synthesis and release of a variety of virulence factors. Despite coordinated gene expression, the ability of a given pathogen to invade host tissue is dependent on the quantity and quality of the virulence factors it produces. Given the heterogeneity of the host immune response, pathogens need to be able to express a variety of virulence factors in large quantities following QSS activation of transcriptional regulators.¹³ Given that virulence factors act synergistically, the pathogen must be able to coordinate the transcription of individual genes to maximize virulence potential. Finally, it must be able to maintain virulence despite changes in the host response. To achieve all of the above, genes responsible for the expression of microbial virulence are housed in discrete genetic units in close proximity to specific sequences of chromosomal DNA (direct repeats, insertion sequences, tRNA genes). These genetic units differentiate pathogenic bacteria from their nonpathogenic counterparts. They are the products of lateral gene transfer and are referred to as pathogenicity islands. These pathogenicity islands represent unstable DNA regions, and changes in their genetic sequences can result in huge clinical consequences. Recently, a genetic alteration involving the pathogenic locus of *Clostridium difficile* resulted in severe cases of *Clostridium difficile*-associated colitis in North America by increasing the strain's toxigenic potential. In addition, these islands may possess gene capture systems (integrons), facilitating the incorporation and dissemination by lateral transfer of antibiotic-resistance genes. A clinically relevant example is the development of a clone of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) with genetic alterations leading to increased toxigenic potential and an epidemic of necrotizing soft tissue infections.

With the aid of virulence factors, pathogens penetrate extraepithelial and epithelial barriers and invade host tissue, establishing infection. Further innate immune system activation occurs with recruitment of immune effector cells to the site of infection, with significant host-pathogen interaction. This recruitment represents the initial significant interaction between the host immune cells and the invading pathogen.

IMMUNOINFLAMMATORY DYSFUNCTION IN SEPTIC SHOCK

Although the dysfunctional events that lead to septic shock involve multiple biologic systems, immune response remains central to the development of septic shock.

Normal Immune Response

The immune system includes a structural component consisting of mucosal barriers to host tissue invasion, a nonspecific early response system (the innate immune response) and a more pathogen-specific response system (the adaptive immune response) activated later following the presence of pathogenic stimuli. Normal immune function requires the coordinated action of these components, resulting in early recognition of a potential pathogen and its subsequent elimination with minimal host tissue damage or disruption to physiologic processes. The structural barriers consist of mucocutaneous membranes (including appendages) and the endogenous

colonizing flora on these surfaces. Optimal function requires proper appendage function and stability of the endogenous flora population.

The innate immune system must be able to recognize invading pathogens early following tissue invasion and mount a response of sufficient intensity to contain the threat. It must also be able to regulate this intense nonspecific response to protect host tissue from injury and facilitate repair.

The adaptive response is charged with “fine-tuning” the later aspects of the immune response. This fine-tuning ensures that, for any given stimulus, the immune response is focused and measured. To understand the degree of host dysfunction and, thus, the pathophysiology of septic shock, one must appreciate certain features of a normal host immune response to microbial infection.

Temporal variation

The normal immune response may be characterized as an initially nonspecific, highly proinflammatory phase, with a subsequent complementary anti-inflammatory response necessary for the restoration of immune homeostasis and prevention of collateral immune-mediated host tissue injury.

Biologic redundancy

In experimental situations, a single pathogenic stimulus triggers the transcription of proinflammatory genetic material, producing a few proinflammatory mediators. In a 1:1 transmission system, the inactivity of any 1 of these pathways can seriously affect the ability of the host to respond adequately to microbial pathogens. To mount effective immune responses a single stimulus to the mammalian innate immune system results in the transcription of hundreds of proinflammatory genes. In addition, different immune effector pathways exhibit pathogenic cross-reactivity with markedly different types of injury stimulating the same pathways. Furthermore, it is likely that, in the clinical setting, there may be multiple injurious stimuli of different durations. The expression of innate immunity becomes biologically redundant and not prone to dysfunction by the inhibition of a few mediators, which protects the system as a whole from being paralyzed by otherwise trivial subunit dysfunction.

Interaction with other biologic systems (cross talk)

The host response to infection extends beyond the immune system to include other biologic systems (coagulation system, autonomic nervous system [ANS]) that interact with the immune system to reduce the potential for host tissue injury, despite a robust immune response, by maintaining organ perfusion (coagulation system) or by appropriately down-regulating the immune response (ANS).

Heterogeneity (genetic and nongenetic)

The immune response to a given pathogen in a given individual is determined by many factors including, but not limited to, the virulence of the pathogen, the individual's genetic composition, and pre-existing comorbidities. Staphylococcal infection of native cardiac valves should elicit a different host immune response from that in response to the common cold, although they both might be febrile illnesses with a cough. After an invading pathogen triggers an immune response, its severity depends on the degree to which the innate immune system is expressed, which in turn depends on genetic and acquired factors. The physiologic response to ongoing infection in the setting of pre-existing comorbidities differs from the response in the otherwise healthy host. Evidence for genetic differences in the immune response is supported by the observation that, with regard to dying from infection, a strong association exists between adoptees and their natural, but not adoptive, parents.⁵ Genetic

polymorphisms in septic shock are discussed elsewhere in the issue of the critical care clinics.

Septic shock is often characterized by dysfunction involving all aspects of the immune response. From a structural standpoint, the disruption may be modifiable and transient (intestinal bacterial overgrowth) versus nonmodifiable factors (mucociliary dysfunction in cystic fibrosis). Immunodysfunction in sepsis may present as an uncontrolled (too intense or too long) early response with subsequent host tissue injury, or as an inadequate response later in the course of the disease. This dysfunction involves cellular and humoral components of the innate and adaptive immune response systems.

Pathogen Recognition

Pattern recognition receptors, pathogen-associated molecular patterns, and danger-associated molecular patterns

The initial event in the innate immune response is the recognition of an invading pathogenic threat. Bacteria and viruses (prokaryotic life forms) have molecular structures that are (largely) not shared with their host, are common to related pathogens, and are invariant. These molecular signatures are also expressed by nonpathogenic and commensal bacteria and, depending on the context, may be referred to as pathogen-associated molecular patterns (PAMPs), or microbial-associated molecular patterns (MAMPs).¹⁴ From a functional standpoint, the endogenous equivalents of these PAMPs are intracellular proteins expressed or released following host tissue injury. These proteins are known as alarmins and, together with PAMPs, are referred to as damage-associated molecular patterns (DAMPs).¹⁵

Immune cells express a set of receptors known as pattern recognition receptors (PRRs) that can recognize and bind to DAMPs expressed by invading pathogens and injured host tissue. At least 4 families of PRRs are recognized: toll-like receptors (TLRs); nucleotide oligomerization domain leucine-rich repeat (NOD-LRR) proteins; cytoplasmic caspase activation and recruiting domain helicases such as retinoic-acid-inducible gene I (RIG-I)-like helicases (RLHs); and C-type lectin receptors expressed on dendritic and myeloid cells.^{16,17} These receptors initiate the innate immune response and regulate the adaptive immune response to infection or tissue injury.

In humans, the TLRs are a family of 10 cell receptors expressed on immune effector cell surfaces and constitute the prototype PRRs; their structure and function illustrate many of the steps involved in initial host-pathogen interaction in sepsis. The TLRs are transmembrane proteins with leucine-rich repeat extracellular domains and an intracellular (cytoplasmic) domain composed of the toll interleukin-1 receptor resistance domain (TIR domain). PAMPs and DAMPs bind to PRRs, such as TLRs, expressed on the surface of host cells. In addition, intracellular PRRs exist and interact with intracellular pathogens, viral particles, and proteins released from damaged tissue (**Fig. 3, Table 1**).

In sepsis, there is a full-blown activation of immune responses due to the release of high levels of DAMPs from invading microorganisms or damaged host tissue, which leads to upregulation of TLR expression. This response has been noted in experimental models and in septic patients.^{18,19} TLR interaction with DAMPs from host tissue injury primes the innate immune system for enhanced TLR reactivity, resulting in excess lipopolysaccharide (LPS)-induced mortality.²⁰ Positive feedback loops between DAMPs/PAMPs and their respective receptors may lead to excessive immunoreactivation, characterized by a markedly imbalanced cytokine response with resultant tissue injury. In contrast, polymorphisms in the TLRs have been linked to increased risks of infection. This association applies equally to polymorphisms in the downstream signaling cascades. Single nucleotide polymorphisms (SNPs)

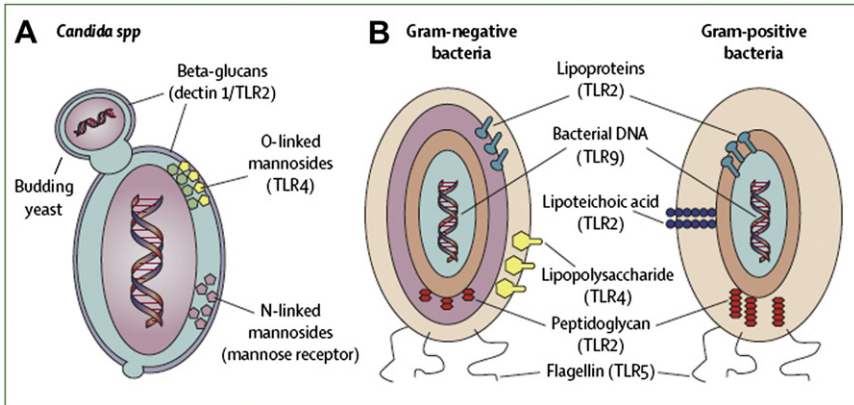


Fig. 3. Innate recognition of pathogens by toll-like (and related) receptors (TLRs). (A) The complexity of the interaction between innate immune receptors and fungi. Three distinct components of the cell wall of *Candida albicans* are recognized by 4 different host receptors: N-linked mannosyl residues are detected by the mannose receptor, O-linked mannosyl residues are sensed by TLR4, and B-glucans are recognized by the dectin 1-TLR2 complex. (B) Gram-positive and Gram-negative bacteria are recognized by partly overlapping and partly distinct repertoire of TLRs. Gram-positive pathogens exclusively express lipoteichoic acid, Gram-negative pathogens exclusively express LPS; common PAMPs include peptidoglycan, lipoproteins, flagellin, and bacterial DNA. (Data from van der Poll T, Opal SM. Host-pathogen interactions in sepsis. *Lancet Infect Dis* 2008;8:35; with permission.)

Table 1 Role of toll-like and other PRRs in the pathophysiology of sepsis		
Pattern Recognition Receptor	Pathogen or Disease State	Relevant PAMPs/DAMPs
TLR 1	Lymedisease <i>Neisseria meningitides</i>	Triacyl lipopeptides
TLR 2	Gram-positive bacteria Mostbacteria <i>Neisseria meningitides</i> <i>Candida albicans</i> Hostproteins (DAMPs)	Lipoteichoic acid Peptidoglycan, triacyl lipopeptides Porins Phospholipomannan, B-glucans HMGB1
TLR-4	Gram-negative bacteria <i>Candida albicans</i> Hostproteins (DAMPs)	Lipopolysaccharide (LPS) D-Mannan, O-linked mannosyl residues Heatshock proteins, fibrinogen, HMGB1
TLR5	<i>Salmonella</i> (flagellated bacteria)	Flagellin
NOD proteins (NOD1 and NOD2)	Gram-negative bacteria	Bacterial peptidoglycan fragments diamino-pimelate (NOD1) and muramyl dipeptide (NOD2)
Cytoplasmic caspase activation and recruiting domain helicases	Viruses	Viral nucleic acids

Data from Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008;36:296–327.

identified in TLR4-CD14 have been linked to LPS hyporesponsiveness.²¹ Higher rates of Gram-negative septic shock were noted in carriers of these SNPs in a medical intensive unit population.²² It is believed that these SNPs predispose affected individuals to endotoxin tolerance with inadequate early expression of proinflammatory cytokines.

Pro- and Anti-inflammatory Cellular Signaling/signal Transduction

Signal transduction describes the sequence of intracellular events in response to the engagement of ligands to their specific receptors (eg, bacterial LPS, cytokines) or changes in the immediate extracellular environment. These molecular interactions trigger the induction of specific cellular responses ranging from the expression of specific gene products (ie, protein production) to adhesion and chemotaxis.

Virtually all intracellular signaling pathways have, as their initial event, activation by phosphorylation of a target protein. Their target proteins could be transcription factors or regulatory (cytoplasmic or nuclear) proteins. This activation is typically accomplished by the binding of an enzyme (a kinase) to the target protein, and can lead to: (1) activation or alteration of its enzymatic activity; (2) changes in the stability of the target protein; (3) subcellular localization of the protein; and (4) interactions with other proteins. Many signaling pathways involve a cascade of 2 or more kinases in series (signaling cascades), involving an upstream kinase involved in enzyme activation and a downstream kinase whose substrate(s) are the protein products of upstream kinase-substrate interaction. Kinase cascades are activated following engagement of ligand-specific receptors (eg, Gram-negative LPS + TLR-4). In addition, certain disruptions of cellular homeostasis (eg, changes in state of oxidation) can lead to activation of kinase cascades.

Kinases are able to phosphorylate other kinases, leading to signal amplification. Thus, a given stimulus may activate multiple kinase cascades, and several kinase cascades may be activated by different stimuli, leading to some measure of redundancy in a cellular signaling pathway. The natural consequence of amplification and redundancy in cellular signaling is a significant degree of overlap and lack of specificity in downstream effects and a requirement for intracellular regulation for effective, host-protective, stimulus-appropriate signal transduction. This negative regulation with resultant transcriptional downregulation is accomplished by dephosphorylation of relevant enzymes leading to a return to baseline levels of activity. Interactions occur between kinase cascades in such a way that increased activity of a given cascade produces suppression of activity in "opposite" cascades. This effect is termed cross talk. In addition, efficient kinase-kinase interaction is facilitated by co-localization of kinases on anchoring or adaptor proteins at relevant intracellular sites. An understanding of the above aspects of cellular signaling is essential to understanding the initial events that occur following host-pathogen interaction in sepsis.

Following the attachment of DAMPs/PAMPs to their specific ligands (TLRS) in severe sepsis, and the subsequent activation of signaling cascades (**Fig. 4**), there is modification of the activity of key intracellular proteins, primarily transcription factors and nuclear and cytoplasmic regulating proteins. For the TLRs, signaling depends primarily on 4 adaptor proteins: the myeloid differentiation primary-response protein 88 (MyD88); and 3 non-MyD88 proteins: (1) toll/interleukin 1 (IL-1) receptor homology (TIR) domain-containing adaptor protein (TIRAP); (2) TIR domain-containing adaptor protein-inducing interferon- β (TRIF); and (3) TRIF-related adaptor molecule (TRAM). These MyD88-dependent and MyD88-independent signal-transduction pathways result in the activation of the prototypical transcription factor, nuclear factor- κ B (NF κ B). In addition, important enzyme systems regulating several key cellular

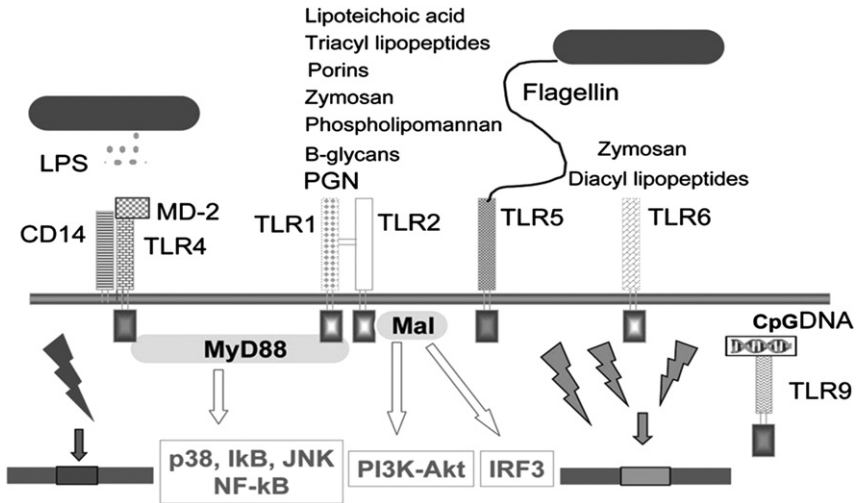


Fig. 4. Binding of toll-like receptors (TLRs) activates intracellular signal-transduction pathways that lead to the activation of transcriptional activators such as interferon regulator factors p13K/Akt, activator protein-1, and cytosolic nuclear factor-kappa[β] (NF- κ)[β]. Activated NF- κ [β] moves from the cytoplasm to the nucleus, binds to transcription sites and induces activation of an array of genes for acute phase proteins, iNOS, coagulation factors, proinflammatory cytokines, and enzymatic activation of cellular proteases. TLR9 DNA, TLR 3 dsRNA, and TLR7/8 ssRNA are endosomal. TLR10 ligand is not defined and TLR1 forms heterodimers with TLR2. LPS, lipopolysaccharide; IRF, interferon regulatory factor; JNK, c-Jun N-terminal kinase. (Adapted from Cinel I, Opal SM. Molecular biology of inflammation and sepsis: a primer. Crit Care Med 2009;37(1):291–304; with permission.)

functions and aspects of cellular signaling (the caspases, phosphoinositide 3-kinase [PI3K] and Rho GTPases) are activated.

NF κ B

NF κ B is the prototypical transcription factor involved in modulating the expression of many of the inflammatory responses associated with severe sepsis and septic shock. It exists as homo- or heterogenous dimers composed of members of the Rel family of proteins (P50, P105, P52, P100, P65 [Rel A], C-Rel). The Rel family of proteins plays pivotal roles in inflammation, and various combinations of NF κ B differ in their degree of transcriptional activity (NF κ B1-P50 + P105, NF κ B2-P52 + P100, Rel A [P65]).

In the absence of cellular activation, NF κ B exists in the cytoplasm maintained in a latent form by interacting with inhibitors of the I κ B family (I κ B- α , I κ B- β , I κ B- γ , I κ B- ϵ , Bcl-3, p100, p105). MyD88-dependent and MyD88-independent kinase pathways activate NF κ B following TLR ligation by diverse stimuli, including bacterial products (LPS, peptidoglycans), cytokines (tumor necrosis factor- α [TNF- α], IL-8, IL-1 β), reactive oxygen species, and changes in the cellular environment, such as ischemia. Regardless of the nature of the stimuli, activation occurs by phosphorylation of I κ B molecules followed by their degradation by the 26S proteasome. This step results in nuclear translocation of NF κ B, its binding to specific gene promoter regions, and gene transcription. NF κ B is involved in the induction of several predominantly proinflammatory gene products (Table 2).

The degree of NF κ B activation in septic patients seems to correlate with patient survival and outcome in septic shock.^{23–25} In addition to the pathophysiologic

Table 2 NFκB-inducible genes involved in sepsis	
Class	NFκB-Dependent Genes
Acute phase proteins	C-reactive protein LPS-binding protein
Cytokines	TNF- α G-CSF, GM-CSF IL-1a, IL-1B, IL-2, IL-6, IL-12 IFN- β
Chemokines	MIP-1 α MIP-2
Coagulation factors	Tissue factor Tissue factor pathway inhibitor
Adhesion molecules	ICAM-1 VCAM-1 E-selectin ELAM-1
Enzymes	Inducible nitric oxide synthase Cyclo-oxygenase-2 C3 complement Phospholipase A2 Matrix metalloproteinases
Immunoreceptors	IL-2 receptor-a Major histocompatibility complex class 1

consequences of excessive NFκB activation, inadequate stimulation can also lead to increased morbidity in septic shock. Studies support the observation that defective NFκB signaling leads to immunosuppression in sepsis, favoring apoptosis in immune effector cells with undesirable consequences.^{26,27} Thus, excessive activation, especially early in the disease course, or excessive negative regulation in sepsis may produce either excess inflammation with collateral host tissue damage or immunoparalysis and consequent direct tissue damage.

The caspases

Caspases are a family of cysteine proteases synthesized as proenzymes and activated by proteolysis. They are further subdivided into initiator caspases, activated by autocleavage, and executioner caspases, activated by cleavage induced by their initiator counterparts. The caspases play important roles in the cellular processes of inflammation and apoptosis following PAMP/DAMP-PRRs interaction. Following cleavage, caspases produce many of the phenotypic changes seen in apoptosis, including cytoskeletal disintegration, DNA fragmentation, and disruption of cellular DNA repair molecular machinery. Although the TLRs are the most studied PRPs, the cytoplasmic NOD-like receptors (NLRs) are the most ubiquitous. Three members of the NLR family (NALP3, ICE protease-activating factor [IPAF], and apoptosis-associated speck-like protein [ASC]) are involved in caspase activation. It is believed that, following pathogen recognition by TLRs, a signal is communicated intracellularly that is recognized by the nucleotide-binding domains of the NLRs. This recognition results in the activation of a multiprotein complex termed an inflammasome. The inflammasomes are multienzyme complexes (>70 KDa) that serve as molecular platforms for the activation of caspases 1 and 5, resulting in caspase-mediated activation and secretion of the proinflammatory cytokines IL-1 β and IL-18. The signals

and mechanisms leading to inflammasome assembly/activation are in general still poorly understood. The inflammasome complexes assembled in sepsis are well characterized and consist of 2 different multiprotein complexes, the NALP1 and NALP3 inflammasomes (Fig. 5). IL-1 β is a highly potent proinflammatory cytokine, requiring inflammasome complex assembly as a prerequisite for caspase-1 activation before its precursor, pro-IL-1 β (p35), released following TLR ligation, can be converted to its active form, which represents a mechanism to prevent uncontrolled expression of IL-1 β . In addition to the release of proinflammatory cytokines, the caspases target the enzyme, caspase-activated DNase (CAD). CAD activation induces DNA fragmentation leading to apoptosis. Cytoskeletal caspase targets include spectrin, nuclear lamin, and the enzyme gelsolin, which cleaves actin.^{28,29} All these play roles in cytoskeletal disintegration. Inflammation-mediated caspase activation contributes to the host response in septic shock. Experiments in murine sepsis models show that the deletion of caspase-1 genes prevents the development of sepsis in affected mice.³⁰

Phosphoinositide 3-kinases

Phosphatidylinositol 3-kinases (PI3Ks) are a group of enzymes that, when activated, catalyze the production of membrane phosphatidylinositol triphosphate (PIP3). PI3K

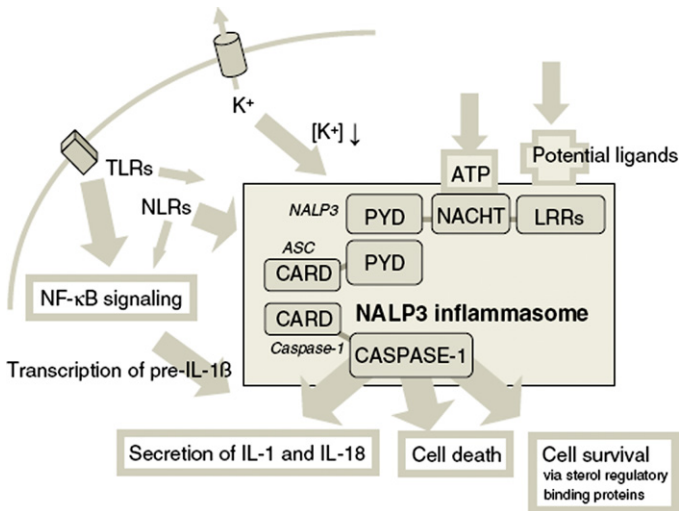


Fig. 5. The NALP3 inflammasome complex. The NALP3 inflammasome is composed of NALP3, ASC, and caspase-1 (a second adaptor protein CADD is present in NALP3 but lacking in NALP1). ASC interacts with 1 of the NALP proteins through Cognate pyrin domain (PYD) interactions and with procaspase-1 through homotypic caspase recruitment domain (CARD) interactions. The human inflammasome complex brings 2 molecules of procaspase-1 (the second via CARDINAL) into close proximity, leading to autocatalysis and the subsequent release of the active catalytic p20 and p10 domains of caspase-1. NALP3 binds ATP via the NACHT (nucleoside triphosphatase [NTPase] domain), is a precursor of IL-1 β via the biologically active fragment, and a potent mediator of fever and inflammation. There is no CARDINAL homolog in the mouse and, hence, murine NALP3 is believed to recruit only a single caspase-1 molecule. (TLRs, toll-like receptors; ATP, adenosine triphosphate; NLRs, nucleotide-binding oligomerization domain (NOD)-like receptors; ASC, apoptosis-associated specklike protein containing a CARD; NALP, NACHT-, LRR-, and PYD-containing protein; LRRs, leucine-rich repeats). (From Trendelenburg G. Acute neurodegeneration and the inflammasome: central processor for danger signals and the inflammatory response? *J Cereb Blood Flow Metab* 2008;28:867–81; with permission).

is activated by a variety of growth factor, hormonal, and chemokine receptors and, together with its downstream counterpart, the serine/threonine kinase Akt (protein kinase B), regulates key aspects of cell proliferation and survival. The downstream targets of PI3K/Akt signaling include direct regulators of neutrophil functioning, including chemotaxis, adhesion, and apoptosis.²⁶ Three isoforms of PI3Ks exist (PI3K- α , PI3K- β , and PI3K- γ) with PI3K- γ found exclusively in leukocytes. PI3K can function either as a positive or negative regulator of TLR signaling and, depending on the cell type or specific TLR involved, activate either the NF κ B or mitogen-activated protein kinase (MAPK) signaling pathways.³¹ The NF κ B signaling pathway has already been reviewed. The MAPK signaling pathway consists of 3 distinct pathways described in leukocytes: p38, extracellular signal-regulated kinase (ERK), and c-jun NH₂-terminal kinase (JNK). These are serine/threonine enzymes that act as the final kinase in a 3-kinase cascade.

The p38 pathway is activated by a broad range of PAMPs (LPS, peptidoglycan) and inflammatory cytokines (tumor necrosis factor [TNF], IL-1, platelet-activating factor), and plays a role in neutrophil cytokine production, adhesion, chemotaxis, respiratory burst, and apoptosis.^{32,33} The ERK pathway is activated by several mitogens (platelet-derived growth factor, insulin, epidermal growth factor, angiotensin II), and in monocyte LPS and cellular adherence. Its primary role is as a regulator of cellular proliferation and differentiation, but it plays significant roles in cytokine (TNF- α) production and chemotaxis to C6a and IL-8. As a positive mediator of TLR signaling, PI3K, together with p38 and ERK, mitogen-activated phosphokinases, leads to production of proinflammatory cytokines IL-1 α , IL-6, and IL-8 on microbial challenge.^{34,35} The JNK pathway is activated by ligand receptor GTPases, cytokines (IL-1), and ultraviolet radiation. This pathway is important in cellular proliferation and apoptosis.

In addition to enabling expression of the immune response, the PI3K/Akt signaling pathway acts as an endogenous negative feedback mechanism that serves to limit proinflammatory and apoptotic events, as seen in monocytes in response to endotoxin.³⁶ It can also promote the generation of anti-inflammatory cytokine IL-10,³⁷ and helps balance Th1 versus Th2 responses.³⁸

The ability of PI3K to modulate events in sepsis in a bidirectional fashion suggests that it could play a role in enhancing the efficacy of the innate immune response and limiting excessive inflammation. This perspective is supported by recent experimental evidence in which, following PI3K gene deletion, mice exposed to a pneumococcal virulence factor developed more neutrophil-mediated alveolar injury despite inadequate alveolar macrophage recruitment.³⁹

Rho GTPases

Cell surface ligand receptors consist of an extracellular ligand-binding domain connected by a single transmembrane region to an intracellular domain that possesses either intrinsic enzyme activity or enzyme activation capabilities. The G-protein-linked family of receptors are the most ubiquitous and are referred to as GTPases (GTP hydrolysis is required for receptor activation and signal transduction). The Rho and Rac subfamilies of GTPases play a central role in the regulation of cell motility by controlling actin cytoskeleton rearrangement following the binding of specific proteases to the cell surface. Following DAMP-PRP interaction, Rho- and Rac-GTPases help regulate the mechanical aspects of the cellular response by the innate immune system. These include cellular migration, pathogen uptake, phagocytosis, and maintenance of endothelial integrity.^{40,41} Rac1 activation is required for PI3K activation on TLR stimulation.⁴²

Release of Pro- and Anti-Inflammatory Mediators

One of the immediate consequences of cellular signaling in severe sepsis and septic shock is the synthesis and release of increased amounts of mediators into the systemic circulation in an attempt to activate more immune effector cells and recruit them to the site of infection. These highly potent molecules are normally present in the circulation in low concentrations, but in high concentrations, or with prolonged exposure, they can exert potentially harmful biologic effects. Over-expression of inflammatory mediators early in the course of sepsis plays a significant role in the eventual development of septic shock. This sequential release of mediators has been termed the cytokine cascade. The availability of precise molecular tools, and the ability to measure cytokine levels, has shed light on the patterns of cytokine release in sepsis, with the earliest cytokines, highly proinflammatory TNF- α and IL-1 β , being responsible for the earliest clinical events in sepsis. The subsequent, and sometimes concomitant, release of counterinflammatory mediators has also been observed. In addition, a clearer understanding of the source, structure, and actions of specific cytokines in the development of septic shock has emerged (**Table 3**), and novel mediators have been discovered. Selected novel cytokine mediators and new ideas regarding the role of otherwise well-known mediators are reviewed below.

High-mobility group box 1 protein

High-mobility group box 1 protein (HMGB1) is a nuclear and cytoplasmic protein originally discovered 3 decades ago as a nuclear binding protein, and was so named because of its rapid mobility on electrophoresis gels. HMGB1 is amongst the most ubiquitous, abundant proteins in eukaryotes, and plays a major role in facilitating gene transcription by stabilizing nucleosome formation. More recent findings suggest that HMGB1 is active in DNA recombination, repair, replication, and gene transcription, facilitated by internal repeats of positively charged domains of the N terminus (HMG boxes). HMGB1 is released passively by necrotic (but not apoptotic) cells, and from macrophages, dendritic cells, and natural killer cells, on activation by microbial pathogens.

The known biologic effects of HMGB1 are based on data obtained from cell cultures. HMGB1 stimulates the release of proinflammatory cytokines, including TNF and IL-8, in macrophages/monocytes and endothelial cells. HMGB1 can also bind to and induce the expression of the cellular receptor for advanced glycation end products (RAGE) and adhesion molecules (vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1]) in human endothelial cells. The induced expression of RAGE facilitates activation of the transcription factor NF κ B and MAPKs. These observations suggest a role for HMGB1 as a proinflammatory cytokine, with significant adverse effects on gut barrier function, and as a regulator of the coagulation system.

At present, HMGB1 does not seem to contribute significantly to the development of septic shock.

Macrophage migration inhibitory factor

Originally described as a T cell product, macrophage migration inhibitory factor (MMIF) is a cytokine produced by various cell types including other immune effector and neuroendocrine cells. MMIF is capable of activating T cells and inducing proinflammatory cytokine production in macrophages. Serum MMIF concentrations are increased in septic patients and, in severe sepsis, elevated MMIF concentrations seem to be an early indicator of poor outcome of septic patients in intensive care.

Table 3
Cytokine and noncytokine mediators of septic shock

Class	Mediator	Source	Role in Septic Shock
Proinflammatory Cytokines	Interleukin-1 β	Monocytes Macrophages Lymphocytes Endothelial cells	Fever, hypotension, T cell and macrophage activation, myocardial suppression
	Tumor necrosis factor- α	Activated macrophages	Fever, hypotension, myocardial depression (myocytes in culture), neutrophil and endothelial cell activation
	Interleukin-6	T cells B cells Endothelial cells	Induction of lymphocyte (B and T cell) proliferation
	Interleukin-8	Activated macrophages and monocytes Kupffer cells	Chemotactic for neutrophils and T cells
	Interleukin-17	Activated T cells	Induces the synthesis of other cytokines IL-6, G-CSF, GM-CSF, IL-1 β , TGF- β , TNF- α and chemokines
	Interleukin-18	Activated macrophages	Alongwith IL-12, initiates the cell-mediated immune response. Increased secretion of interferon- γ
	Interferon- γ	Natural killer cells	Defense against viral and intracellular bacterial pathogens
	Macrophage inhibitory factor/macrophage migration inhibitory factor (MIF)	Activated macrophages	Increased TNF expression Increased TLR4 expression

Anti-inflammatory cytokines	Interleukin-10	Epithelial cells Monocytes Lymphocytes	Downregulation of macrophage function leading to decreased TNF- α
	IL-4 IL-1Ra TGF-B	? Monocytes Various host cells	Induces differentiation of naive helper T cells to Th2 cells Block IL-1 activity Interferes with phagocytic activation
Endothelial Factors	Nitric oxide		Increased microvascular permeability Loss of vasomotor tone Myocardial depression Peripheral venous pooling
Hormones	Vasopressin Glucocorticoids	Posterior pituitary gland Hypothalamic-pituitary axis	Relative deficiency may cause or worsen circulatory failure
Arachidonic acid metabolites	Prostaglandins Leukotrienes Thromboxanes	Immune effector cells Pancreas	Airway reactivity Vasoconstriction Platelet aggregation Increased vascular permeability
Others	Platelet-activating factor	Endothelial cells Macrophages Neutrophils	Histamine release from platelets Activation of endothelial cells
	Complement proteins C3a-C5a		Histamine release, Increased capillary permeability, vasodilation
	Myocardial depressant factors	Pancreas	Negative inotropy Impaired phagocytosis

Based on our present understanding, MMIF does not seem to play a significant role in the development of septic shock.

Immune and Nonimmune Effector Cell Dysfunction

The normal immune effector response in response to cytokine release is lost in severe sepsis. The dysfunctions can involve every cell type from antigen-presenting cells (dendritic cells) to neutrophils and macrophages. The nature of cellular dysfunction is similar to the associated cytokine homeostatic imbalance with elements of increased cellular activity and cellular hyporesponsiveness.

Neutrophil dysfunction in severe sepsis

Neutrophils are key cells in the innate immune system and act primarily by recognizing and destroying pathogens by a coordinated series of steps including adhesion, chemotaxis, phagocytosis, and the release of cytotoxic molecules, followed by apoptotic cell death with the neutrophils spending approximately 7 hours in the systemic circulation. In severe sepsis the tight regulation of neutrophil function is lost, leading to excessive neutrophil activation and prolonged survival. These activated neutrophils induce endothelial dysfunction, release cytotoxic molecules, and lead to inflammatory host organ injury.

Accelerated lymphocytic apoptosis

The T-helper lymphocytes play key roles in the adaptive immune response following activation by antigen-presenting cells of the innate immune system. Following activation, the initial lymphocyte response is proinflammatory, with the emergence of a regulatory phenotype after several days. Severe sepsis is characterized by accelerated apoptotic death of lymphocytes leading to lymphocyte depletion and loss of the T lymphocyte regulatory function.⁴³

In addition to immune effector dysfunction, there is widespread nonimmune cellular dysfunction in severe sepsis. The alterations relevant to the development of cardiocirculatory failure in the setting of severe sepsis are discussed in a later section.

Interactions with Other Biologic Systems in Severe Sepsis

The coagulation cascade in severe sepsis

As much as 4 decades ago, it was apparent that the coagulation system was abnormally activated in septic patients,^{44–46} and, by 1970, Dr James Corrigan⁴⁷ had published on the potential use of anticoagulant therapy (heparin) to treat disordered coagulation in sepsis. Almost all patients with septic shock have coagulation abnormalities. The nature and degree of coagulation dysfunction ranges from clinically silent biochemical evidence of dysfunction to full-blown disseminated intravascular coagulation (DIC). An understanding of the hemostatic system, including the blood coagulation pathways and the natural anticoagulant pathways, is necessary for any meaningful review of sepsis-induced coagulation dysfunction.

Although the classic model of coagulation (extrinsic and intrinsic pathways) may be helpful in the interpretation of commonly used laboratory tests of coagulation disorders, it does not represent a clinically relevant model of hemostasis in physiologic and, more importantly, pathophysiologic states such as sepsis. Current understanding of hemostasis describes the coagulation pathway as a 3-phase process (initiation, amplification, and thrombin action), with considerable overlap between the phases. These phases are counterbalanced by active natural anticoagulant systems targeting key steps in the coagulation cascade. The result is a hemostatic system that begins to form a clot less than 30 seconds following

vascular injury, with the process of thrombolysis initiated as soon as a thrombus is formed.

Initiation occurs shortly after vascular injury and is the result of increased tissue factor (TF) expression by TF-bearing adventitial cells and platelets. Limited amounts of TF bind to and activate factor VII (FVII). The TF-FVIIa complex leads to production of a limited amount of thrombin.

Amplification consists of thrombin activating platelets and other coagulation cofactors during the amplification phase. There is release of significant amounts of prothrombin (factor X) and, together with platelets and the other coagulation factors, a prothrombinase complex (factor Xa and coagulation factors bound to activated platelets) is formed and is primarily responsible for the burst of thrombin production leading to the third phase of clot formation.

The final phase of coagulation involves thrombin-dependent recruitment of additional coagulation factors (FV and FXIII) into the coagulation process, maintaining platelet activation and facilitating the conversion of fibrinogen to fibrin.

The natural anticoagulant systems serve to prevent clot formation in the absence of vascular injury and prompt lysis of clots formed following vascular injury.

The balance between procoagulant and anticoagulant arms of the hemostatic process is disrupted in severe sepsis. The inflammatory response to severe infection results in a systemic dysfunction of the coagulation system. The events that constitute coagulation dysfunction in septic shock can be divided into an initial activation, followed by a largely dysregulated response with suppression of the antifibrinolytic systems. Cytokines released as part of the inflammatory response mediate many of the hypercoagulable responses triggered in severe sepsis and septic shock, and the available evidence suggests contributory roles for immune effector cells, endothelial dysfunction, and metabolic alterations in the tissue. It is now understood that, in sepsis, the interaction between the coagulation and inflammatory systems is bidirectional (**Fig. 6**). Binding of coagulation proteases (thrombin or TF) or anticoagulant proteins (activated protein C [APC]) to specific cell receptors on mononuclear cells or endothelial cells may affect cytokine production or inflammatory cell apoptosis. Endothelial cells respond to the cytokines expressed and released by activated leukocytes, but they can also release cytokines themselves. Endothelial cells are able to express adhesion molecules and growth factors that promote the inflammatory response and also affect the coagulation response. Endothelial cells play a prominent role in all 3 major pathogenetic pathways associated with coagulopathy in sepsis: TF-mediated thrombin generation, dysfunctional anticoagulant pathways, and inhibition of fibrinolysis.

APC acts in conjunction with the cofactor protein S to deactivate clotting factors Va and VIIIa, preventing ongoing thrombin generation by the prothrombinase complex. APC may also inhibit inflammation by inhibiting cytokine production, preventing neutrophil activation, and inhibiting leukocyte adhesion and rolling. Other key mediators in the hypercoagulable cascade in sepsis include antithrombin and TF pathway inhibitor.

This complex cross talk between the inflammatory and coagulation cascades represents a vicious cycle which, if uninterrupted, results in tissue injury, organ dysfunction, and cellular death.

Neural regulation of the immunoinflammatory response: the vagal inflammatory reflex and sympathetic effects on the host response

In addition to the humoral mechanisms that act to prevent tissue injury from excessive release of proinflammatory mediators, emerging research suggests a role for neural modulation of inflammation. One of the earliest documented

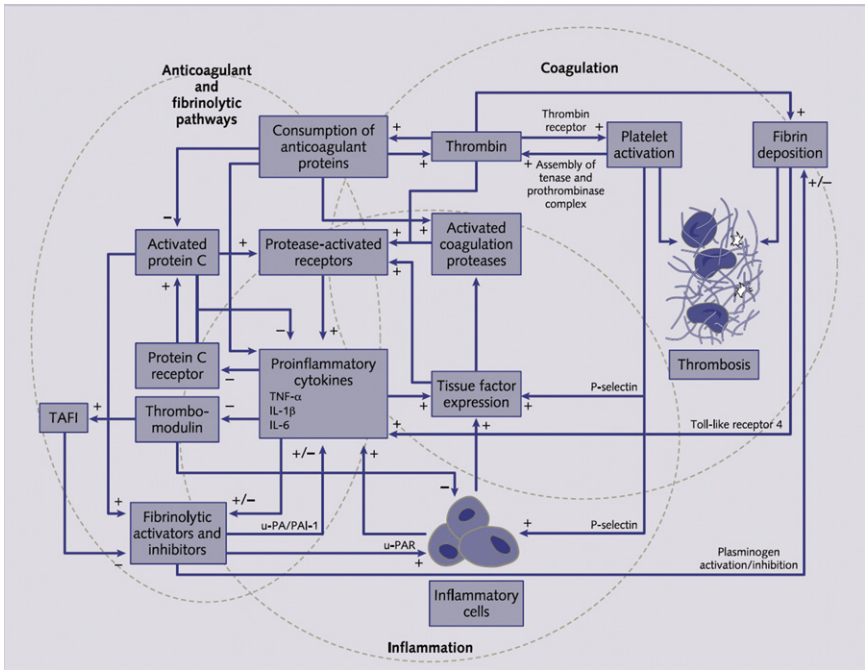


Fig. 6. Schematic overview of the major pathways involved in the interrelation between coagulation, anticoagulant pathways, and fibrinolysis and inflammation. +, stimulatory effect; -, inhibitory effect; IL, interleukin; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin activatable fibrinolysis inhibitor; TNF, tumor necrosis factor; u-PA, urinary plasminogen activator; u-PAR, urokinase-type plasminogen activator receptor. (From Levi M, van der Poll T. The central role of the endothelium in the crosstalk between coagulation and inflammation in sepsis. *Adv Sepsis* 2004;3:93; with permission).

observations supporting the existence of central autonomic interaction with the immunoinflammatory response involved a serendipitous finding that, with central administration of a TNF inhibitor, efferent vagal activity was stimulated with systemic anti-inflammatory action.⁴⁸

Subsequent work in animal sepsis models demonstrated significant inhibition of TNF expression following vagal stimulation and improved disease end points in these models.^{49,50} In addition, rendering these animals immune to vagal stimulation either by genetic manipulation or vagotomy led to an exaggerated proinflammatory cytokine response.⁵¹ It is now understood that these cytokine-suppressive effects of vagal stimulation are mediated by the release of its neurotransmitter acetylcholine (ACh) and its subsequent interaction with ACh receptors expressed by macrophages and other immune effector cells.^{52,53} The best characterized of these cholinergic receptors that suppress cytokines is the $\alpha 7$ subunit of the nicotinic acetylcholine receptor ($\alpha 7$ nAChR).

This autonomic parasympathetic-mediated immune modulation system has been termed the inflammatory reflex (**Fig. 7**), with an immunosensing afferent arm (cytokine stimulation of vagal afferents) and an efferent immunosuppressing arm (the cholinergic anti-inflammatory pathway).⁵⁴ Recent studies suggest that the spleen plays a significant role as an effector organ for this pathway.

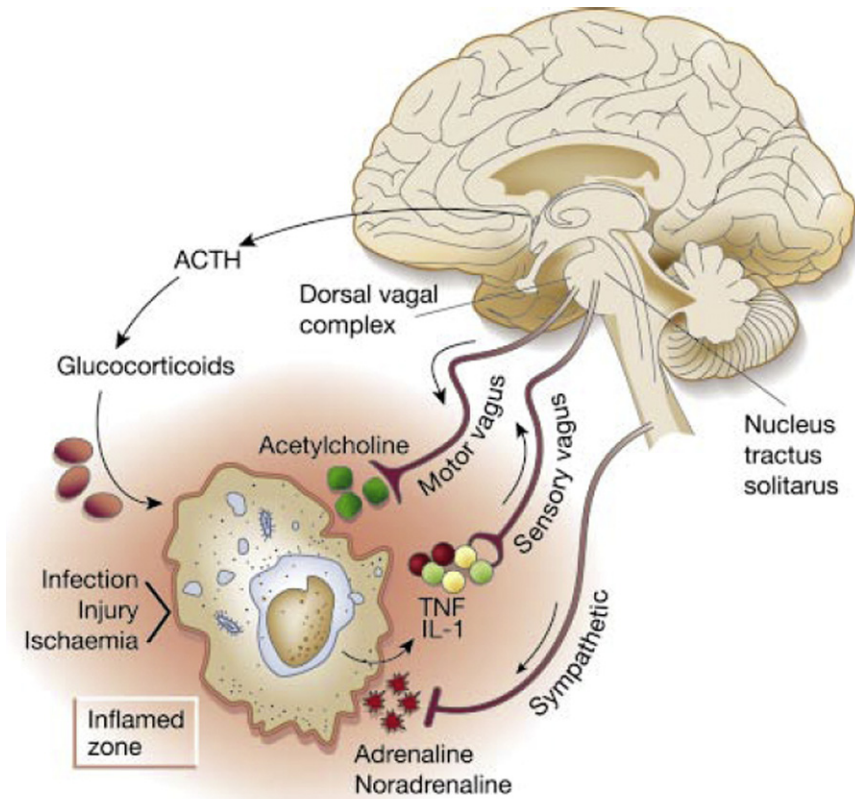


Fig. 7. Wiring of the inflammatory reflex. Inflammatory products produced in damaged tissues activate afferent signals that are relayed to the nucleus tractus solitarius; subsequent activation of vagus efferent activity inhibits cytokine synthesis through the cholinergic anti-inflammatory pathway ('the inflammatory reflex'). Information can also be relayed to the hypothalamus and the dorsal vagal complex to stimulate the release of ACTH, thereby activating the humoral anti-inflammatory pathway. Activation of the sympathetic outflow by flight-or-fight responses or pain, or through direct signalling, can increase local concentrations of adrenaline and noradrenaline, which can suppress inflammation further. (Data from Tracey KJ. The inflammatory reflex. *Nature* 2002;420:857).

The sympathetic ANS consists of sympathetic neurons and the adrenal medulla, with catecholamines as the neurotransmitter. In addition to the adrenal medulla and sympathetic neurons, immune effector cells are also a source of catecholamines in severe sepsis.⁵⁵ Early uncomplicated sepsis is characterized by high circulating catecholamine levels with significant metabolic (catabolic state), immunomodulatory (excessive inflammation), and cardiocirculatory (increased cardiac output) consequences. Prolonged elevation of circulating catecholamines is toxic to host cells, predisposing the patient to cardiocirculatory failure with hypotension resulting from peripheral vasodilatation and compromised myocardial contractility.⁵⁶ Septic shock is more often characterized by depletion of endogenous catecholamine stores and, possibly, catecholamine resistance.

CARDIOCIRCULATORY DYSFUNCTION IN SEVERE SEPSIS RESULTING IN PROGRESSION TO SEPTIC SHOCK

The widespread disruptions in severe sepsis can result in cardio-circulatory dysfunction manifesting as shock. The dysfunction involves the cardiac, peripheral vascular (macrovascular) and microcirculatory elements of the circulation and, depending on the degrees of cardiac or vascular dysfunction and the volume status of the patient, a clinical picture ranging from cold, clammy and under-perfused to one of hyperdynamic shock, may be seen, although, in clinical medicine, hyperdynamic shock is seen much more frequently. The situation in septic shock is further complicated by widespread microcirculatory dysfunction, further impairing tissue oxygen delivery, and diminished mitochondrial activity resulting in impaired oxygen extraction. A review of characteristics and pathogenetic mechanisms that underlie cardiac and macrovascular dysfunction in septic shock follows. The microcirculatory alterations are discussed elsewhere in this issue.

Over 5 decades, multiple methods of myocardial function assessment have been used to study ventricular performance in severe sepsis and septic shock.⁵⁷⁻⁶⁰ The results have been largely similar and the characteristic pattern of cardiac performance during septic shock has been proved to be one of reduced left and right ventricular ejection fractions, increased end-diastolic and end-systolic volumes of both ventricles, and normal stroke volume; heart rate and cardiac output are elevated, and systemic vascular resistance is reduced. The reduction in the ejection fraction and the biventricular dilatation occur 24 to 48 hours after the onset of sepsis and, like most other organs affected by the septic process, it is reversible with restoration of myocardial function if patients survive up to 10 days after their onset. An inability to maintain cardiac output during this critical period is associated with a poor outcome,⁶¹ and ventricular dilatation allows for an increased end-diastolic volume and helps maintain cardiac output. Thus, the decrease in ejection fraction with ventricular dilatation in septic shock may be an appropriate adaptive response to myocardial dysfunction.

Myocardial depression results from the direct or indirect effects of 1 or more circulating myocardial depressant substances. In experiments, ultrafiltrates from patients with severe sepsis show cardiotoxic effects.⁶² Several of the cytokines released in severe sepsis probably contribute to this dysfunction.

TNF- α and IL-1

TNF- α and IL-1 are associated with myocyte dysfunction and may explain the early myocardial depression seen in sepsis. In one series of studies using in vitro myocardial cells and human septic shock serum, TNF and IL-1 were shown to be responsible for the myocardial depressant activity present in human sera.⁶³ These cytokines are potent inducible nitric oxide synthase (iNOS) inducers, and this probably represents one of the direct mechanisms for myocardial depression.

Nitric Oxide

Severe sepsis is associated with increased expression of iNOS and increased nitric oxide production. NO interferes with myocyte calcium metabolism and may impair contractile function. In addition, reactive nitrogen species such as peroxynitrite, produced by NO interacting with superoxide ions, are directly toxic to myocytes. Experimental observations support a role for NO-mediated myocardial depression in sepsis. Cardiac function was preserved following LPS challenge in iNOS-deficient mice.⁶⁴

Vascular Dysfunction

Vascular alterations in septic shock are mainly due to the effects of mediators on vascular smooth muscle and endothelial dysfunction.

The NO released seems to be primarily responsible for vascular smooth muscle dysfunction in sepsis. NO causes a hyperpolarization of smooth muscle plasma membranes, rendering them unresponsive to catecholamines and causing a vasodilatory state. In addition to the above, these patients may have relative vasopressin or cortisol deficiencies, leading to further catecholamine unresponsiveness and refractory shock.

Endothelial dysfunction leads to an inability of the endothelial cells to maintain vascular tone with loss of blood pressure. In addition, endothelial damage leads to capillary leak with intravascular volume depletion and edema formation in involved organs.

SUMMARY

Septic shock remains a significant challenge for clinicians. Recent advances in cellular and molecular biology have significantly improved our understanding of its pathogenetic mechanisms. These improvements in understanding should translate to better care and improved outcomes for these patients.

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