Immunopathogenesis of Sepsis: New Insights

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Sepsis is a result of a complex network of events. The initiation of sepsis is triggered by the components of the cell wall (endotoxin) of Gram negative bacteria. Following the generation of pro- and anti-inflammatory mediators, both cellular and humoral immune system are activated. Three major pathways are activated which are able to interact with each other; complement system, the inflammatory response (recruitment of white blood cells), and the coagulation and fibrinolysis pathways. The pathophysiology of sepsis and the identification of genes involved in immune response against infectious agents could contribute to understanding of the molecular basis of susceptibility to sepsis and the different forms of clinical manifestations of sepsis and to offer more effective therapies. New potential therapeutic drugs are under investigation with increased knowledge of the pathophysiology of sepsis.


Keywords: immunopathogenesis, sepsis, cytokines

Sepsis is a disease characterized by an overwhelming systemic response to infections with bacteria, viruses, fungi, protozoa or rickettsiae which can rapidly lead to organ dysfunction and ultimately death. Severe sepsis is considered as the most common cause of death in non-coronary care units, with about 150 000 people dying every year from this disease process in countries of the European Union. This review highlights the latest developments in immunopathogenesis of sepsis and focus on the potential new therapeutic agents in the treatment of sepsis¹⁻⁴

Definitions:
Bacteremia is defined as a positive blood culture result. The child with bacteremia usually appears only mildly ill. It may be a transient phenomenon and not be associated with disease.

Sepsis is the systemic response to an infection manifested by hyperthermia (temperatures >38° C or 100.4° F) or hypothermia (temperatures < 36° C or 96.8° F), tachycardia (heart rate > 160/min in infants; > 150 / min in children ).

Septic shock is defined as sepsis with hypotension [systolic blood pressure in infants <65 m Hg; in children: < 75 mm Hg (or below the fifth percentile for age)] and hypoperfusion.

Sepsis can strike anyone, and can be triggered by events such as pneumonia, trauma, surgery and burns or noninfectious etiologies as well. In the newborn period the most common pathogens are, group B Streptococcus and Escherichia coli. Fulminant sepsis may develop following Listeria monocytogenes, Enterobacteriaceae, Staphylococcus aureus, coagulase negative staphylococci, and Enterococcus infections.

27
Candida albicans and viral infections may mimic clinical manifestations of sepsis. The organism usually associated with fulminant sepsis in children beyond 8 weeks of age is Neisseria meningitidis.

There are many predisposing risk factors leading to bacteremia in infants and children:
* Agammaglobulinemia
* Acquired Immunodeficiency Syndrome (AIDS)
* Asplenia
* Chemotherapy
* Complement and properdin deficiency
* Congenital heart disease
* Extensive burns
* Foreign body material
* Instrumentation of respiratory, gastrointestinal, or genitourinary tract
* Intractable diarrhea of infancy
* Intravascular or invasive monitoring device
* Malignancy
* Multiple trauma
* Neutropenia
* Prematurity
* Sickle cell disease
* Surgery
* Transplantation
* Urinary tract malformation

**Clinical manifestations:**
Bacterial sepsis is manifested through a two-phase process; hypermetabolic and hypometabolic phases. The initial phase is characterized by fever, chills, tachypnea, cutaneous lesions (petechiae, erythema, later on purpura fulminans), changes in mental status such as confusion, lethargy or coma, increased cardiac output and tissue perfusion, enhanced metabolic rate, and hyperglycemia and hyperinsulinemia, due to pro-inflammatory mediators. The hypometabolic phase, also known as septic shock, is associated with marked immune suppression which leads to depressed organ function and subsequent death.

Laboratory findings correlating with clinical status:
* Hemotologic: Anemia, altered white blood cell count, elevated neutrophil and band count.
* Coagulation: Prolonged prothrombin time, prolonged partial tromboplastin time, presence of fibrin split products or D-dimers.
* Inflammatory: Increased serum concentrations of C-reactive protein, procalcitonin, reduced fibrinogen levels.
* Hemodynamic: Increased cardiac output, low systemic vascular resistance, low oxygen extraction ratio
* Metabolic: Hyponatremia, hypocalcemia, hypoalbuminemia, elevated serum lactate, creatinine phosphokinase, serum alanine and aspartate aminotransferase levels, hyperbilirubinemia, increased insulin requirements
* Altered tissue, and skin perfusion, reduced renal blood flow and urinary output
* Organ failure: Increased urea and creatinine, low platelet count and/or dysfunction, other coagulation abnormalities.

**Immunopathogenesis**
Sepsis is a result of a complex network of events. The initiation of sepsis is triggered by the components of the cell wall (endotoxin) of Gram negative bacteria. In addition to endotoxins other bacterial molecules may also contribute to the generalized response in sepsis. After invasion of the bloodstream by the microbial components, three major pathways; the complement system, the inflammatory response (recruitment of white blood cells), and coagulation and fibrinolysis pathways are activated. These pathways do not act independently but are able to interact with each other. Following the generation of pro- and anti-inflammatory mediators, both cellular and humoral immune systems are triggered. These mediators consist of cytokines, coagulation factors, adhesion molecules, myocardial depressor substances, and heat shock proteins.
Figure 1. Pathogenetic Network in Sepsis
Although the cellular immune response to bacterial toxins normally protects the host against infection, overactivation of the cellular immune system may cause serious injury. Under normal conditions, there are specific inhibitors strictly suppressing the biological activity of the mediators involved in sepsis. This balance is disrupted during sepsis leading to profound alterations in the relative production of a variety of mediators. Therefore, the pathogenesis of sepsis may also called a “pro- and anti-inflammatory disequilibrium syndrome”.

Neutrophils

Neutrophils are generally the first cells to arrive at the site of inflammation and bone marrow releases more than usual number of neutrophils (leukocytosis) in response to infections. Chemotactic factors promote accumulation of neutrophils at the site of inflammation. Neutrophils have been regarded as double-edged swords in sepsis. Neutrophils and macrophages ingest bound microbes into their vesicles where the microbes are destroyed. Toll-like receptors (TLRs), G protein-coupled, Fc and C3 receptors and receptors for cytokines recognize microbes and function cooperatively to activate neutrophils. Activation of NADPH oxidase induces reactive oxygen intermediates (ROIs) that destroy microbes in phagolysosomes. When neutrophils are strongly activated, excessive release of ROIs, nitric oxide (NO) and lysosomal enzymes such as proteases, phospholipases, elastases, collagenases can injure normal host tissue if these products enter the extracellular environment.

Toll-like receptors

The toll family of receptors play an essential role in innate immunity, recognizing molecular components such as LPS, endotoxin, bacterial DNA, unmethylated CpG nucleotides, peptidoglycans, and mycolic acids which are found only in bacteria. Ten different TLRs have been identified and each TLR recognizes a different class of infectious pathogen. TLRs also have ability to stimulate and modulate the course of adaptive immunity, thus a temporary blockade of toll-like receptors may serve to treat or prevent sepsis that involve inappropriate adaptive immune responses. Interruption of TLR signaling at the early stage of pathogen recognition is shown in CD14-deficient mice. Those mice were resistant to the toxicity of LPS as well as Gram-negative sepsis. Anti-CD14 monoclonal antibodies provided significant protection in humans against sepsis. However, antibiotics must be administered along with anti-CD14 monoclonal antibodies for adequate protection. In another recent study, resistance to lethal doses of LPS has been demonstrated in mice lacking IRAK-4, a protein involved in the cytoplasmic propagation of TLR signals. Mutations in the Toll-like receptor 4 gene (TLR4) have been identified in humans who are more susceptible to infections. Some studies have shown that TLRs are using the same signaling molecules used by the interleukin-1 receptors (IL-1Rs) including MyD88, IL-1R-associated protein kinase and tumor necrosis factor receptor-activated factor 6. However, there is evidence suggesting that the signaling pathways associated with each TLR are not identical and may result in different biological responses. Therefore, although endotoxin has deleterious effects, total blockade of endotoxin may be detrimental. Reasons for the failure of monoclonal anti-endotoxin antibodies to improve outcomes in trials involving patients with sepsis are complex.

Endothelial cells

Endothelial cell damage accounts for much of the pathology leading to septic shock. Circulating bacterial molecules encounter with vascular endothelial cells immediately at the site of entry. Endothelial cells are capable of recognizing structural components of bacterial pathogens subsequently initiating the generation of inflammatory mediators. Endothelial cells predominantly express TLR-4 and very low levels of TLR-2 and the expression of both of these TLR’s is regulated by LPS, tumor necrosis factorα, (TNF) and interferon, (IFN)
Complement pathway

Another protein involved in innate immune system in sepsis is the complement cascade. Three pathways play a key role in the complement cascade:

* Classic
* Alternative
* Lectin

All cascades aim to form the membrane attack complex. During this process a number of ancillary mediators, such as C3, C4, C5 are produced. They release small cationic peptides that bind to G-protein coupled receptors on different cells. The activation of these receptors leads to leukocyte chemotaxis, release of granule bound enzymes and cytokines. Some studies showed that in vivo neutralization of C5a with specific anti serum is largely protective in the model of sepsis. An excess of intravascular C5a, down regulates C5a receptors. Neutralization of C5a can protect against the sepsis syndrome. As a result of the effects of C5a infusion on polymorphonuclear neutrophils the immune responses to C5a and other chemoattractants are suppressed. Administration of antibodies against complement-activation product C5a decreased the frequency of bacteremia, prevented apoptosis and improved survival. It is considered that the effects of C5a mediated by cellular C5a receptors and that blockade of this G- protein coupled receptors may be a therapeutically valuable process.

Cytokines

Research by a number of investigators suggests that the main pro-inflammatory mediators produced during an infection, are tumor necrosis factor-α (TNF-α), IL-1β, IL-1, IL-12, IFN-α, IL-6 and IL-18. They transmit danger signals, which alert the various components of the host defense.

It is suggested that the pro-inflammatory cytokines, particularly TNF-α, IL-2, IL-12, IFN-γ, and IL-6 may be the ones initiating cell and organ dysfunction leading to sepsis and multiple organ failure (MOF). However, the levels of pro-inflammatory cytokines are low during the early stages of sepsis, possibly reflecting the host's initial response to the invading bacteria. In the later stages of sepsis, the capability of splenic lymphocytes to produce IL-1 and IFN-γ is significantly depressed whereas they can generate increased amounts of anti-inflammatory cytokines such as IL-4 and IL-10. It is suggested that the anti-inflammatory response resulting with immunosuppression in combination with the injury inflicted by inflammation leads to deleterious conditions such as MOF or septic shock. Finally, the pathogenesis of sepsis is complex consisting of a number of cellular and mediator interactions and the mechanisms controlling this network needs to be further investigated.

Sepsis looks like a “cytokine anarchism”. The innate immune system recognizes pathogen-associated molecular patterns and generates activation signals. Downstream from activation of the members of the TLR, IL-1, TNF-α, IL-6, and IL-8 are synthesized, released and activate leukocytes. In certain forms of sepsis for example, meningococcemia, circulating TNF levels are high and correlate with mortality. Understanding of the cell-signaling pathways that mediate the response to microbes have demonstrated that the concept of blocking endotoxin in order to prevent septic complications may be simplistic.

These studies used large doses of endotoxin or bacteria; consequently, levels of circulating cytokines such as tumor necrosis factor (TNF) were exponentially higher in animals than they were in patients with sepsis.

T cells

Sepsis causes lymphopenia which is inversely correlated with patient survival. The role of apoptosis-specific immune-activation and activation-induced cell-death in sepsis is incompletely understood. In a study, fifteen septic patients and 20 healthy controls were included, T-cell proliferation was measured by [3H]thymidine uptake. Apoptosis and cell phenotype were determined by FACS. sTNFR1, sCD95, interleukin-1β converting enzyme (sICE), and interleukin -10 were measured by ELISA. PHA and CD3-driven T-
cell proliferation were significantly decreased in septic patients. The percentages of CD3+ and CD4+ T cells and CD19+ B cells were significantly reduced. The percentage of memory T-cells (CD45RO+) and cells undergoing apoptosis (CD95+/annexin-V+) were significantly increased in sepsis. Moreover, sCD95, sTNFRI, and ICE were significantly increased. Anti-CD3 antibody triggering induced a 56% increase of CD4 T-cell death in septic patients vs. 7.5% in controls relative to IgG. Serum level of IL-10, a Th2 cytokine was increased. These findings strongly suggest that in septic patients Th1 cells are selectively susceptible to undergo apoptosis. This observation provides an additional pathophysiological concept in the genesis of Th2 dominance.

Stimulation of the release of other cytokines and chemokines, notably Th1 cytokines, such as interferon-γ (IFN-γ), lymphotoxin α (LTα) and IL-2, results in the activation of the microbicidal potential of neutrophils and macrophages, mainly through the production of reactive oxygen and nitrogen species. Recruitment of phagocytes and lymphocytes at the site of infection, upregulation of cellular receptors for the pathogenic microorganisms and their products and potentiation of phagocytosis are additional mechanisms triggered by pro-inflammatory cytokines to combat the invading microorganism.

Coagulation Pathways:
The coagulation cascade, fibrinolysis, and inflammation are controlled by the factors listed below during sepsis:

* Inflammation is activated
* Coagulation is activated
* Fibrinolysis is suppressed
* Homeostasis is lost

Primary factors leading to organ dysfunction and death are, pro-thrombotic and anti-thrombotic mechanisms that maintain hemostasis (Figure 2). Imbalance in hemostatic mechanisms in sepsis is manifested as:

* Intravascular coagulopathy (DIC)
* Microvascular thrombosis
* Acute multiple organ dysfunction

Activated Protein C
The coagulant and inflammatory exacerbation in sepsis is counter balanced by the protective protein C pathway. Activated protein C was shown to use the endothelial cell protein C receptor as a co-receptor for cleavage of protease activated receptor-1 in endothelial cells. Gene profiling demonstrated that protease activated receptor-1 signaling could account for all activated protein C induced protective genes, including the immunomodulatory monocyte chemoattractant protein-1 which was selectively induced by activation of protease activated receptor. The prototypical thrombin receptor is the target for the endothelial cell protein C receptor-dependent activated protein C signaling, suggesting a role for this receptor cascade in protection from sepsis. Endogenous activated protein C has direct anti-inflammatory actions, such as decreasing cytokine production and inhibiting leukocyte attachment to the endothelium. Endogenous activated protein C has been shown to limit the production of TNF-α and IL-1 and interfere with the interaction between lipopolysaccarides and CD14. Endogenous activated protein C may therefore work to reinstate the balance in all three of the major processes causing sepsis: coagulation, suppressed fibrinolysis, and inflammation (Figure 1). An uncontrolled cascade of coagulation, impaired clot breakdown, and inflammation promotes the progression of sepsis. Leukocytes, platelet activating factor, oxygen free radicals, may also lead to tissue damage, organ failure, and subsequent death. The theory that death from sepsis was attributable to an over-stimulated immune system was based on studies in animals that do not seem to reflect the clinical picture in humans. Both the intrinsic and extrinsic blood coagulation pathways, the generation of thrombin and serum protease are crucial steps during coagulation. The pro-coagulation cascade is in balance with anti-coagulation factors, such as antithrombin, a plasma protein protease inhibitor, and protein C, all of which cleave tissue factors involved in the pro-coagulation process.
Figure 2. The Coagulation Cascade in Sepsis
Genetic factors
In recent years, significant progress has been made in human genomics and host inflammatory response. It is demonstrated that the immune response to infection is dependent on host gene polymorphisms. In other words, host genetic factors determine susceptibility to infectious diseases in humans. The polymorphisms of cytokine genes (TNF-α, TNF-β, IL-1-ra) have been reported to influence the concentration of secreted mediators and to modify the regulation of the inflammatory cascade inversely. Mutations of Fc gamma receptor, toll like receptor or mannose binding protein have been shown to lead severe pneumococcal infections, septic shock by Gram-negative bacteria, and meningococcal disease. Hubacek et al have demonstrated the gene variation by analyzing the polymorphism (Cys98 to Gly, Pro436 to Leu) of the LPS-binding protein in septic patients with poor outcome. Coagulation cascade is also affected in patients with sepsis due to gene variants such as of the plasminogen activator inhibitor-1 (PAI-1). The functional polymorphism of PAI-1 increases the risk of death from meningococcal infection or severe trauma. Thus, at any step of the host immune response to infectious agents may be affected by genetic factors and these genetic variants have been associated with susceptibility and/or poor outcome of severe sepsis and septic shock.

The Nervous System
The Nervous System regulates the inflammatory response on a reflexive basis. Inflammatory products produced in damaged tissues activate afferent signals that are relayed to the nucleus tractus solitarius; subsequent triggering of the activity of the afferent vagus inhibits cytokine synthesis through the cholinergic anti-inflammatory reflex (the inflammatory reflex). Information can also be delivered to the hypothalamus and the dorsal vagal complex to stimulate the release of ACTH, thereby activating the humoral anti-inflammatory pathways. The activation of the sympathetic outflow by flight-or-fight responses or pain, or through direct signaling, can increase local concentrations of adrenaline and noradrenaline, which can further suppress inflammation (Figure 3).

Current Concepts and Potential Therapies for Sepsis
The lack of an apparent acute-phase response in patients with sepsis is associated with high mortality and may reflect the immunosuppressive phase of sepsis. Early manifestations of sepsis include subtle changes in mental status, minor increases or decreases in white cell count or neutrophil percentage, or elevated blood glucose levels. Early recognition of sepsis is a key for successful treatment. The individual response is determined by many factors, including the virulence of the organism, the size of the inoculum, and the patient’s coexisting conditions, age, and polymorphisms in genes for cytokines. The initial immune response to pathogen is hyperinflammatory, but the response rapidly progresses to hypoinflammatory phase. Serum concentrations of inflammatory mediators may be useful in evaluating the stage of sepsis, and for the decision of anti-inflammatory agents. Immune-enhancing therapy such as interferon may be efficacious to improve survival, instead of anti-inflammatory drugs which may worsen the outcome during the hypoimmune phase. It is demonstrated that Interferon can activate macrophages by restoring macrophage HLA-DR expression in patients with sepsis. Evaluation of the spleens removed after the death of patients with sepsis demonstrated that the more prolonged the sepsis, the more profound was the loss of B cells and CD4 T cells. Most deaths occurred during the prolonged hypoimmune state, and reversal or prevention of this immune deficiency should be a major focus of research. Anti-inflammatory strategies applied early in patients with a hyperinflammatory immune response may be life-saving.
Figure 3. Inflammatory Reflex
Diverse new agents have shown efficacy in clinically relevant animal models. Interleukin-12, a potent immune stimulant and Th1 inducer, reduced mortality from subsequent sepsis when administered after burn injury. High concentrations of macrophage inhibitory factor were present in patients with sepsis and the administration of antibodies against macrophage migration inhibitory factor protected mice from peritonitis. Strategies that block apoptosis of lymphocytes or gastrointestinal epithelial cells have improved survival in experimental models of sepsis. Mice with sepsis that are deficient in poly-ADP-ribose polymerase 1 (PARP) have improved survival and administration of a PARP inhibitor was beneficial in pig models. The central nervous system is an important modulator of inflammation; electrical stimulation of the vagus nerve protects against endotoxic shock. TNF, interleukin-1 and high-mobility group 1 protein which are the late mediators of the lethality in mice were well correlated with outcome in patients with sepsis.

**Insulin Therapy for Hyperglycemia**

The protective mechanism of insulin in sepsis is unknown. The phagocytic function of neutrophils is impaired in patients with hyperglycemia, and correcting hyperglycemia may improve bacterial phagocytosis. Another potential mechanism involves the antiapoptotic effect of insulin. Insulin prevents apoptotic cell death from numerous stimuli by activating the phosphatidylinositol 3-kinase-Akt pathway.

**Corticosteroids**

Administration of high doses of corticosteroids does not improve survival in patients with sepsis and may worsen outcomes by increasing the frequency of secondary infections.

In conclusion, the latest developments in pathophysiology of sepsis and the identification of genes involved in immune response against infectious agents may contribute to understanding of the molecular basis of susceptibility to sepsis, different forms of clinical manifestations of sepsis and to offer more effective therapies. New potential therapeutic drugs are under investigation with increased knowledge of the pathophysiology of sepsis. These new drugs are proposed to interrupt the sepsis cascade and enhance or inhibit the patient’s immune response.

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