Published in final edited form as:

Eur J Anaesthesiol Suppl. 2008 ; 42: 146–153.

Advances in understanding sepsis

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Summary

Sepsis, a systemic inflammatory response to infection, is a leading cause of death in intensive care units. Recent investigations into the pathogenesis of sepsis reveal a biphasic inflammatory process. An early phase is characterized by pro-inflammatory cytokines (e.g. tumour necrosis factor- α), whereas a late phase is mediated by an inflammatory high-mobility group box 1 and an anti-inflammatory interleukin-10. Inflammation aberrantly activates coagulation cascades as sepsis progresses. This dual inflammatory response concomitant with dysregulated coagulation partially accounts for unsuccessful anti-cytokine therapies that have solely targeted early pro-inflammatory mediators (e.g. tumour necrosis factor- α). In contrast, activated protein C, which modifies both inflammatory and coagulatory pathways, has improved survival in patients in severe sepsis. Inhibition of the late mediator high-mobility group box 1 improves survival in established sepsis in pre-clinical studies. In addition, recent advances in molecular medicine have shed light on two novel experimental interventions against sepsis. Accelerated apoptosis of lymphocytes has been shown to play an important role in organ dysfunction in sepsis and techniques to suppress apoptosis have improved survival rate in sepsis models. The vagus nerve system has also been shown to suppress innate immune response through endogenous release and exogenous administration of cholinergic agonists, ameliorating inflammation and lethality in sepsis models.

Keywords

SEPSIS; INFLAMMATION; APOPTOSIS; COAGULATION; IMMUNOSUPPRESSION; CHOLINERGIC FIBRES

A classical view: sepsis as a TNF-mediated inflammatory disorder and failures in anti-TNF treatment

Sepsis is the generalized inflammatory response elicited by an infectious process. Severe sepsis is manifested by organ dysfunction (i.e. hypoperfusion, tissue hypoxia, lung injury, etc.), while septic shock is a type of severe sepsis marked by hypotension despite fluid resuscitation [1, 2]. Treating patients with severe sepsis and septic shock has been a great challenge to intensive care specialists. Despite advances in supportive treatments, the mortality and morbidity of septic shock remain high. More than 75 000 patients die of septic shock every year in the US, making this syndrome the leading cause of death in non-coronary ICUs and the tenth most common cause of death overall. The incidence of sepsis increases in patients who are elderly, immune-compromised and undergoing invasive procedures [2]. Since sepsis continues to be a substantial burden on healthcare, it is essential not only to elucidate the pathogenesis but also to develop effective treatments.

The classical view regarding the pathogenesis of sepsis results from hyper-inflammatory responses that mediate the excessive production of cytokines (e.g. tumor necrosis factor- α (TNF- α)) and aberrantly activate the immune system. Both lead to tissue injury and organ dysfunction [1,3,4]. Inflammation is a self-limiting and protective response of the body in which cytokine production is under control and leucocytes are properly activated to eliminate infectious agents. However, aberrant innate immune response to infection has been implicated into triggering dysregulated hyper-inflammatory responses in sepsis [1,3,4]. Recognition of bacterial products (e.g. lipopolysaccharide (LPS)) by toll-like receptors (TLRs) on macrophages/monocytes leads to the production of pro-inflammatory cytokines, chemokines and nitric oxide [5]. Engagement of TLRs elicits signalling cascades via the activation of nuclear factor κ B (NF- κ B). TNF- α plays a pivotal role in the pathogenesis of an early shock state (i.e. hypotension, fever) and at least part of organ dysfunction related to septic shock [1, 3,4]. TNF- α provokes neutrophil-mediated tissue injury by acting on endothelial cells and neutrophils. TNF- α enhances the expression of adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) as well as chemokines in endothelial cells. TNF- α also activates neutrophils by up-regulating integrin adhesiveness and promoting extravasation to the lungs, liver, gut and so forth. Extravasated neutrophils damage tissues by releasing oxygen free radicals and proteases. In addition, TNF- α amplifies inflammatory cascades in an autocrine and paracrine manner by activating macrophages/monocytes to secrete other pro-inflammatory cytokines.

In the classical view of sepsis, TNF- α constitutes the critical mediator of sepsis, thus supporting the hypothesis that neutralization of TNF- α could ameliorate it [2,6]. Blocking monoclonal antibodies (mAbs) to TNF- α ameliorated shock and improved survival in LPS-induced experimental septic shock models. Suppression of other pro-inflammatory mediators (interleukin-1 (IL-1), platelet-activating factor and nitric oxide) yielded similar results in pre-clinical investigations [2]. However, when tested in sepsis patients under controlled clinical trials, few anti-pro-inflammatory therapies improved survival, except for the one [7] that modestly reduced mortality rates in a subset of patients with increased IL-6 levels. Rather, inhibition of TNF- α with a high dose of a TNF-receptor-Fc fusion protein worsened patient survival, possibly by inducing an iatrogenic immune-defect that exacerbated infections [8].

In contrast to sepsis, anti-TNF treatments showed clinical efficacy in other inflammatory diseases such as Crohn's disease [9] and rheumatoid arthritis [10], suggesting that sepsis is not merely an inflammatory disease but that it involves a distinct pathophysiology. Failures in clinical trials of anti-pro-inflammatory mediator therapies for sepsis suggest the dauntingly complex pathogenesis, which involves a delicate balance between inflammation, anti-inflammation and coagulation [2,6]. This at least partly explains why anti-mediator therapies that target only inflammation (i.e. anti-TNF- α) have failed to improve survival. In addition, recent investigations into sepsis have revealed the key roles of the late mediators, aberrant apoptotic cell death pathways and neuronal regulation of innate immunity. In this article, we review recent advances in the understanding of pathogenesis as well as potential therapeutic targets in sepsis.

Recent advances in sepsis

Crosstalk between inflammation and coagulation

Complex interactions between inflammation and coagulation are involved in the pathogenesis of sepsis [11,12]. Patients with sepsis exhibit platelet activation, up-regulation of pro-coagulation pathways and down-regulation of anti-coagulation pathways. These abnormal coagulation pathways lead to the formation of micro-vascular thromboses that compromise tissue perfusion and give rise to organ dysfunction. Inflammation in sepsis skews the balance of haemostasis to the pro-coagulation states. In endothelial cells, neutrophils and monocytes,

TNF- α and IL-1 induce the expression of tissue factor, which is typically exposed by sub-endothelial cells upon vascular injury. The aberrant expression of tissue factor by TNF- α initiates blood coagulation cascades. Tissue factor then binds to factor VIIa (FVIIa) in circulating blood, thereby forming a tissue factor-FVIIa complex that activates FX to FXa. The latter subsequently converts prothrombin to thrombin. Thrombin, a serine protease, converts fibrinogen to fibrin, which is further stabilized by inter-molecular cross-linking via a transglutaminase FXIII similarly activated by thrombin. In fact, thrombin activates other coagulation factors such as FXI, FV, FVIII and FXIII. In addition, thrombin activates platelets, monocytes and endothelial cells through thrombin receptors. Thus, thrombin acts to form a positive feedback loop that can amplify coagulation as well as other inflammatory cascades.

The thrombin pathway serves as a negative feedback loop that dampens coagulation [11,12]. Protein C, a plasma protein synthesized in the liver and that circulates in the blood as an inactive zymogen, plays a critical role in this negative feedback process. Protein C is converted by thrombin to its active form, activated protein C (APC) and is deposited onto the surface of endothelial cells to form the thrombin-thrombomodulin—endothelial protein C receptor complex. APC with its co-factor protein S inactivates FVa and FIIIa, thereby negatively regulating blood coagulation. Defects in the negative feedback by APC can lead to hyper-coagulation states. A genetic protein C deficiency is known to exhibit micro-vascular thrombosis [13]. A missense mutation in FV, Arg506Gln, which generates FV resistance to APC-induced proteolytic inactivation, is associated with an increased risk of venous thrombosis [14].

In addition to acting as an antithrombotic factor, APC possesses anti-inflammatory properties [11,12]. It inhibits the production of inflammatory cytokines and the transduction of NF- κ B signalling in monocytes stimulated by LPS and thrombin [15]. APC also suppresses endothelial permeability as well as the transendothelial migration of neutrophils [16]. Therefore, APC plays a pivotal role in the negative regulation of coagulation and inflammation. In patients with sepsis, the following observations have been made: serum concentration of protein C is decreased by inflammation [17], the conversion to APC is reduced [18] and decreased protein C level is associated with poorer outcomes [19]. Furthermore, the administration of APC was shown to have protective effects in a baboon model of lethal *Escherichia coli* sepsis [20].

In the controlled clinical trial 'Protein C Worldwide Evaluation in Severe Sepsis (PROWESS)', APC (Drotrecogin Alfa (Activated); Eli Lilly and Co., Indianapolis, IN, USA) decreased mortality in those sepsis patients with a low risk of bleeding: 24.7% compared to 30.8% in the placebo-treated group [21]. This result generated considerable excitement: APC became the first specific treatment in a phase III clinical trial to show benefits in patients with severe sepsis. However, the initial enthusiasm greeting APC as a magic bullet for sepsis gradually died down, as further analysis of patient sub-groups showed that APC was beneficial only to the sickest patients, i.e. those with severe sepsis and a high risk of death (APACHE Score equal to or more than 25). A subsequent clinical trial involving severe sepsis patients with a low risk of death (the Administration of Drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS)) was terminated early after an interim analysis found no significant benefits or effectiveness [22]. This study suggested that APC might not be beneficial to low-risk patients.

Identification of a late mediator HMGB1

Kinetic analysis of cytokine production in sepsis has revealed that plasma levels of TNF- α and IL-1 peak at the early stage and decrease to undetectable levels in the late stage [1,23]. Despite symptoms of shock during the early stage (e.g. hypotension, fever), most instances of mortality in experimental sepsis, as well as in sepsis patients, occur in the late stage. This partially explains why late neutralization of TNF- α , an early mediator of sepsis, failed to halt its progression in clinical trials.

While searching for mediators that play a critical role in the late phase of sepsis, Tracy and colleagues identified the cytokine HMGB1 (high-mobility group box 1) [24]. In an LPS-induced mouse sepsis model, TNF- α peaked 2 h after injection of LPS. However, serum HMGB1 levels began to increase 12-18 h after TNF had peaked. Inhibition of HMGB1 by a specific mAb protected mice from mortality in both LPS-induced and caecal ligation and puncture (CLP)-induced sepsis, even when the antibody was administered after TNF had peaked. Furthermore, administration of recombinant HMGB1 protein recapitulated severe sepsis by inducing lethal organ dysfunction.

Before its re-discovery as a cytokine that mediates lethality in the late phase of sepsis, HMGB1 was originally identified as a chromatin-binding protein in the nucleus that associated with chromosomal DNA [23]. HMGB1 exists ubiquitously in the nucleus of all eukaryotic cells, where by binding to DNA and several transcription factors it plays a critical role in stabilizing nucleosome formation and in regulating transcription. HMGB1 is released from the nucleus to the extracellular space when cells undergo necrotic cell death [23,24]. As the membrane integrity is disrupted during necrosis, HMGB1 is passively leaked into the extracellular space where it elicits inflammation. Of note, however, is the fact that apoptotic cells do not release HMGB1 extracellularly, since HMGB1 is sequestered within the nucleus and prevented from release. The differing ability of necrotic and apoptotic cells to release HMGB1 supports the idea that necrotic, but not apoptotic, cells evoke inflammation. In addition to passive release, HMGB1 is actively secreted from macrophages and monocytes upon activation with TNF.

The HMGB1 released into the extracellular space, either from necrotic cells in damaged tissue or from activated macrophages at sites of infection, generates signals that activate immune cells and induce inflammation. It has been proposed that HMGB1 acts as a member of damage-associated molecular pattern molecules (DAMPs), together with S100 and heat shock proteins [23]. Extracellular HMGB1 signals via cell surface receptors such as Receptor for Advanced Glycation End-products (RAGE) and TLR2 and TLR4. Extracellular HMGB1 activates macrophages through RAGE and TLR2/4 to secrete TNF- α and other pro-inflammatory cytokines, thereby amplifying inflammatory cascades [23,25]. By binding to RAGE on endothelial cells, HMGB1 up-regulates ICAM-1 and VCAM-1 expression, and has been implicated in the enhanced accumulation of leucocytes [26]. Furthermore, HMGB1 increases the permeability of gut epithelial cells [27]. Thus, in addition to sustaining inflammation by acting on monocytes and macrophages, the induction of endothelial and epithelial damage could constitute a mechanism of HMGB1 released during sepsis to evoke organ dysfunction.

Several techniques have been developed to inhibit the biological activity of HMGB1 in sepsis. A protein fragment A-box, which contains the DNA-binding domain of HMGB1, competes with intact HMGB1 for binding to RAGE [28]. As a competitive inhibitor, A-box has exhibited a therapeutic effect in sepsis models even when administered after the onset of the diseases. Ethyl pyruvate, a stable and non-toxic derivative of an endogenous anti-oxidant pyruvic acid, has been shown to not only suppress HMGB1 release from macrophages *in vitro* but also reduce serum HMGB1 levels and improve survival in sepsis models [29]. The anti-inflammatory effects of ethyl pyruvate are at least partly explained by its ability to interfere with the NF- κ B pathway. Ethyl pyruvate is currently being tested against sepsis in pre-clinical settings. In addition, it is being evaluated in a phase II clinical trial to study the protective effects to patients undergoing major cardiac surgery with cardiopulmonary bypass [30].

Apoptosis of lymphocytes to cause immunosuppression

Sepsis is characterized by a surge of the pro-inflammatory cytokines TNF- α and IL-1 at the early stage. However, as the disease progresses, the hyper-inflammatory state during the early stage coverts to the anti-inflammatory state, marked by decreased levels of TNF and increased levels of IL-10 [1,2,31]. IL-10 inhibits TNF- α production by macrophages and suppresses

expression of costimulatory molecules in macrophages and T-cells. The increased production of IL-10 in the late phase of sepsis is believed to contribute to 'immunosuppression' [31]. Although some patients die of cardiopulmonary collapse in this hyper-dynamic state, many survive and progress into the later stage, a hypo-dynamic state in which IL-10 dominates TNF. Immunosuppression in the late stage of sepsis exacerbates those bacterial infections that are refractory to extensive antibiotic treatment. In addition to increased levels of IL-10, depletion of immune cells by apoptosis has recently emerged as a potential pathological mechanism for immunosuppression in sepsis [32,33].

Apoptosis or programmed cell death constitutes an actively controlled 'cell suicide', in which intrinsic machinery triggers intracellular signalling cascades that lead to DNA degradation (i.e. enzymatic fragmentation of the nucleosome) [32,34]. The other form of cell death necrosis represents an 'accidental cell death' whereby extrinsic insults disrupt cellular integrity, thus killing cells independently of the intrinsic cellular programme. Apoptosis was primarily implicated as a mechanism to remove unnecessary cells following physiological cell death, such as occurs during involution. However, growing evidence has shown that it plays an important role in a wide range of other pathological cell death settings as well.

Similar to a coagulation pathway involving a cascade of zymogen activation reactions, the apoptosis pathway utilizes a family of serine proteases known as caspases, which form intracellular signalling cascades. They in turn lead to the activation of a Dnase (caspase-activated Dnase, CAD), which executes DNA fragmentation. At least two major caspase cascades have been identified in apoptosis [32,34]. The first cascade is initiated by engaging 'cell death receptors' (e.g. CD95 (Fas), TNF-receptor) by cognate ligands (CD95L (Fas-L), TNF), leading to the activation of caspase 8. The other cascade is initiated from the 'mitochondria' in cytoplasm. Cellular stresses such as reactive oxygen and growth factor deprivation trigger release of the pro-apoptotic cytochrome c, which activates caspase 9. Activation of caspase 8 in the 'cell death receptor' cascade and of caspase 9 in the 'mitochondria' cascade converges to activate caspase 3, which in turn leads to the activation of CAD. Activated CAD carries out the apoptosis programme, cleaving DNA into nucleosomes.

Apoptosis of lymphocytes has been observed both in animal models and in autopsies of patients who have died from sepsis [32,33]. The extent of apoptosis correlates with the severity of the disease [35]. Lymphocytes are critical for protecting against polybacterial sepsis; e.g. lymphocyte-deficient Rag-1 knockout mice showed decreased survival compared to wild-type mice [36]. Depletion of lymphocytes is believed to compromise the immune system's ability to control infection, thereby contributing to increased morbidity in sepsis. In addition, the presence of apoptotic cells was shown to cause other immune cells to become anergic. Adoptive transfer of apoptotic, but not necrotic, splenocytes increased mortality in CLP mice [37]. This effect was not observed in interferon- γ -deficient mice. Dendritic cells (DC) are a potent inducer of antigen presentation. In contrast to necrotic cells, ingestion of apoptotic cells by DC failed to stimulate DC to induce costimulatory molecule expression [38], which could lead to anergy. The accelerated apoptotic cell death of lymphocytes is thought to be responsible for immunosuppression in sepsis. Enhanced apoptosis has also been observed in vascular endothelial cells [39] and gut epithelial cells [40] under conditions of sepsis. Epithelial and endothelial cell apoptosis is thought to be involved in the genesis of organ dysfunction in sepsis.

Several experimental approaches for suppressing apoptosis protect mice from sepsis lethality, further supporting a causative role of apoptosis for the pathogenesis and/or progression of sepsis [32,33]. *gld* mice that have a defect in the death receptor ligand CD95L are less susceptible to sepsis [41]. Transgenic over-expression in lymphocytes of bcl-2, a mitochondrial membrane protein, which antagonizes the apoptosis induced by many triggers, not only

conferred lymphocytes a resistance to apoptosis under septic conditions but also improved survival [36]. Recently, it has been reported that the transgenic over-expression of Akt, another anti-apoptosis molecule that functions independently of bcl-2, also decreased sepsis-induced lymphocyte apoptosis and reduced lethality [42]. Caspase inhibitors have been used to block apoptosis as a therapeutic antagonist to sepsis. The broad-spectrum caspase inhibitor zVAD.fmk improved survival in a CLP sepsis model [43]. The protective effects afforded by caspase inhibition were further supported by experiments using a selective caspase-3 inhibitor [44], as well as a siRNA knockdown of caspase 8 [45]. These results demonstrate that an anti-caspase strategy represents a promising novel treatment for sepsis. Nonetheless, a potential drawback is that caspase inhibition might cause hyper-acute TNF-induced shock [46]. Of note is the fact that APC has been shown to possess anti-apoptotic effects that could contribute to its clinical benefits [47,48]. APC might constitute an alternative therapeutic approach for inhibiting apoptosis.

Neuronal regulation of inflammation

Crosstalk between the central nervous system (CNS) and the immune system was originally postulated more than two decades ago [49]. Recent investigations have revealed the possibility that communication between the CNS and immune system, via the vagus nerve, can be exploited to suppress inflammation in sepsis [50,51]. The vagus nerve is a component of the autonomic nervous system that supplies parasympathetic innervations to major organs including the heart, lung and visceral organs. Increasing evidence supports the contention that the vagus nerve also provides innervations to the reticuloendothelial system to monitor and regulate innate immune responses. Inflammation at the periphery is sensed by the CNS through humoral and afferent neural routes [50]. The inflammatory cytokines TNF- α and IL-1 circulating in the blood were shown to gain access to the CNS, where they caused symptoms of sickness (e.g. fever, chills, malaise and anorexia) [52]. Alternatively, the vagus nerve that expresses the IL-1 receptor mRNA and enables IL-1 binding could directly sense inflammatory cytokines and signal this to the brain [53].

In response to relayed signals indicating peripheral inflammation, the CNS in turn signals to the periphery via the vagus nerve to modify inflammation. Stimulation of the vagus nerve has been shown to suppress macrophage function using the main parasympathetic neurotransmitter acetylcholine (ACh) [54]. Macrophages express the $\alpha 7$ subunit of the nicotinic ACh ($\alpha 7$ -nACh) receptor [55]. The cholinergic agonists nicotine and ACh inhibit release of TNF- α as well as HMGB1 from LPS-stimulated macrophages [54,56]. Interestingly, cholinergic agonists do not block the secretion of anti-inflammatory IL-10 from macrophages. One example of a molecular mechanism by which cholinergic agonists inhibits pro-inflammatory cytokine release from macrophages involves the engagement of the $\alpha 7$ -nACh receptor on macrophages, which causes interference with LPS-stimulated NF- κ B signalling [57].

The anti-inflammatory effects of cholinergic agonists have been shown to be effective *in vivo*. Vagotomy, a surgical interruption of the vagus nerve, augmented TNF production and exacerbated the progression of shock in endotoxemia in rats [58]. In contrast, direct activation of the efferent vagus nerve with electric stimulation attenuated TNF- α release during endotoxemia and protected animals from shock [54]. Suppression of TNF by electric stimulation was not observed in $\alpha 7$ -nACh receptor-deficient mice, confirming the brief that anti-inflammation requires the $\alpha 7$ -nACh receptor. Nicotine, a selective cholinergic agonist, inhibited HMGB1 release and improved survival in established sepsis in a mouse CLP model [56]. The protective effects against sepsis stemming from electrical nerve stimulation and a cholinergic agonist were abolished by either splenectomy or selective surgical denervation of the vagus nerve branch to the spleen. This demonstrated the importance of the spleen in the cholinergic anti-inflammatory pathway [59].

In addition to macrophages, endothelial cells have been shown to express the $\alpha 7$ -nACh receptor. They may pose a target for the cholinergic anti-inflammatory pathway [57]. Stimulation of endothelial cells with a cholinergic agonist CAP55 suppressed the TNF-induced expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin via inhibition of the NF- κ B pathway. Furthermore, electrical vagus nerve stimulation in tandem with the cholinergic agonist inhibited leucocyte migration to a site of inflammation in a mouse air pouch model.

Concluding remarks: the intersection between supportive and anti-mediator treatments

Recent advances in sepsis research have revealed the complex pathophysiology, which involves early and late mediators, accelerated apoptotic cell death, aberrant activation of coagulation and the altered neuronal regulation of immune cells. These new findings constitute a foundation for developing novel therapeutic approaches that interfere with specific mediators in sepsis. While it is imperative to advance our understanding of the pathogenesis of sepsis and to develop specific therapeutics for severe sepsis, the benefits of early goal-directed treatment (EGDT) to sepsis have not only shown the importance of early initiation of supportive therapies but also illustrated how supportive therapies potentially impact on inflammatory and coagulatory pathways. EGDT attempts to reverse persistent global tissue hypoxia in sepsis by employing a comprehensive supportive treatment that reverses early (~6h) haemodynamic perturbations [60]. Patients who received EGDT showed significantly reduced late mortality (~60 days) in comparison to those who received a standard therapy. More importantly, the reversal of global tissue hypoxia in sepsis by EGDT reduced pro-inflammatory and pro-coagulatory responses (i.e. decreased plasma IL-8 and D-dimer levels, respectively) [61]. Bearing that notion, it is intriguing to speculate that the benefits of EGDT are due at least in part to its immuno-modulatory effects. Further investigations are needed to determine whether or not therapeutic interventions with specific mediators could result in synergistic or additive effects in concert with the benefits obtained by reversing global tissue hypoxia in sepsis.

Acknowledgments

We thank Dr Sulpicio G. Soriano and Dr Christopher V. Carman for critically reading the manuscript. This work is supported by NIH grant AI63421 (M.S.).

References

1. Russell JA. Management of sepsis. *N Engl J Med* 2006;355:1699–1713. [PubMed: 17050894]
2. Rice TW, Bernard GR. Therapeutic intervention and targets for sepsis. *Annu Rev Med* 2005;56:225–248. [PubMed: 15660511]
3. Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Ann Intern Med* 1996;125:680–687. [PubMed: 8849154]
4. Kox WJ, Volk T, Kox SN, Volk HD. Immunomodulatory therapies in sepsis. *Intensive Care Med* 2000;26:S124–S128. [PubMed: 10786969]
5. Lakhani SA, Bogue CW. Toll-like receptor signaling in sepsis. *Curr Opin Pediatr* 2003;15:278–282. [PubMed: 12806257]
6. Reinhart K, Karzai W. Anti-tumor necrosis factor therapy in sepsis: update on clinical trials and lessons learned. *Crit Care Med* 2001;29:S121–S125. [PubMed: 11445746]
7. Panacek EA, Marshall JC, Albertson TE, et al. Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab')₂ fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Crit Care Med* 2004;32:2173–2182. [PubMed: 15640628]
8. Fisher CJ Jr, Agosti JM, Opal SM, et al. The Soluble TNF Receptor Sepsis Study Group. Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein. *N Engl J Med* 1996;334:1697–1702. [PubMed: 8637514]

9. Lichtenstein GR, Yan S, Bala M, Blank M, Sands BE. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. *Gastroenterology* 2005;128:862–869. [PubMed: 15825070]
10. Genovese MC, Becker JC, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005;353:1114–1123. [PubMed: 16162882]
11. Zeerleder S, Hack CE, Willemin WA. Disseminated intravascular coagulation in sepsis. *Chest* 2005;128:2864–2875. [PubMed: 16236964]
12. Slofstra SH, ten Cate H, Spek CA. Signal transduction induced by activated protein C: no role in protection against sepsis? *Trends Mol Med* 2006;12:374–381. [PubMed: 16820322]
13. Marlar RA, Mastovich S. Hereditary protein C deficiency: a review of the genetics, clinical presentation, diagnosis and treatment. *Blood Coagul Fibrinolysis* 1990;1:319–330. [PubMed: 2103316]
14. Dahlback B, Villoutreix BO. Regulation of blood coagulation by the protein C anticoagulant pathway: novel insights into structure-function relationships and molecular recognition. *Arterioscler Thromb Vasc Biol* 2005;25:1311–1320. [PubMed: 15860736]
15. Yuksel M, Okajima K, Uchiba M, Horiuchi S, Okabe H. Activated protein C inhibits lipopolysaccharide-induced tumor necrosis factor- α production by inhibiting activation of both nuclear factor- κ B and activator protein-1 in human monocytes. *Thromb Haemost* 2002;88:267–273. [PubMed: 12195699]
16. Zeng W, Matter WF, Yan SB, Um SL, Vlahos CJ, Liu L. Effect of drotrecogin alfa (activated) on human endothelial cell permeability and Rho kinase signaling. *Crit Care Med* 2004;32:S302–S308. [PubMed: 15118535]
17. Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. *Chest* 1992;101:816–823. [PubMed: 1531791]
18. Boehme MW, Deng Y, Raeth U, et al. Release of thrombomodulin from endothelial cells by concerted action of TNF- α and neutrophils: *in vivo* and *in vitro* studies. *Immunology* 1996;87:134–140. [PubMed: 8666425]
19. Boldt J, Papsdorf M, Rothe A, Kumle B, Piper S. Changes of the hemostatic network in critically ill patients - is there a difference between sepsis, trauma and neurosurgery patients? *Crit Care Med* 2000;28:445–450. [PubMed: 10708181]
20. Taylor FB Jr, Chang A, Esmon CT, D'Angelo A, Vigano-D'Angelo S, Blick KE. Protein C prevents the coagulo-pathic and lethal effects of *E. coli* infusion in the baboon. *J Clin Invest* 1987;79:918–925. [PubMed: 3102560]
21. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;344:699–709. [PubMed: 11236773]
22. Abraham E, Laterre PF, Garg R, et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. *N Engl J Med* 2005;353:1332–1341. [PubMed: 16192478]
23. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005;5:331–342. [PubMed: 15803152]
24. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248–251. [PubMed: 10398600]
25. Yu M, Wang H, Ding A, et al. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock* 2006;26:174–179. [PubMed: 16878026]
26. Fiuza C, Bustin M, Talwar S, et al. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. *Blood* 2003;101:2652–2660. [PubMed: 12456506]
27. Raman KG, Sappington PL, Yang R, et al. The role of RAGE in the pathogenesis of intestinal barrier dysfunction after hemorrhagic shock. *Am J Physiol Gastrointest Liver Physiol* 2006;291:G556–G565. [PubMed: 16751175]
28. Yang H, Ochani M, Li J, et al. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proc Natl Acad Sci USA* 2004;101:296–301. [PubMed: 14695889]
29. Ulloa L, Ochani M, Yang H, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. *Proc Natl Acad Sci USA* 2002;99:12351–12356. [PubMed: 12209006]

30. Aneja R, Fink MP. Promising therapeutic agents for sepsis. *Trends Microbiol.* 2006
31. Scumpia PO, Moldawer LL. Biology of interleukin-10 and its regulatory roles in sepsis syndromes. *Crit Care Med* 2005;33:S468–S471. [PubMed: 16340424]
32. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. *Nat Rev Immunol* 2006;6:813–822. [PubMed: 17039247]
33. Wesche DE, Lomas-Neira JL, Perl M, Chung CS, Ayala A. Leukocyte apoptosis and its significance in sepsis and shock. *J Leukoc Biol* 2005;78:325–337. [PubMed: 15817707]
34. Adrain C, Brumatti G, Martin SJ. Apoptosomes: protease activation platforms to die from. *Trends Biochem Sci* 2006;31:243–247. [PubMed: 16595176]
35. Hotchkiss RS, Osmon SB, Chang KC, Wagner TH, Coopersmith CM, Karl IE. Accelerated lymphocyte death in sepsis occurs by both the death receptor and mitochondrial pathways. *J Immunol* 2005;174:5110–5118. [PubMed: 15814742]
36. Hotchkiss RS, Swanson PE, Knudson CM, et al. Over-expression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. *J Immunol* 1999;162:4148–4156. [PubMed: 10201940]
37. Hotchkiss RS, Chang KC, Grayson MH, et al. Adoptive transfer of apoptotic splenocytes worsens survival, whereas adoptive transfer of necrotic splenocytes improves survival in sepsis. *Proc Natl Acad Sci USA* 2003;100:6724–6729. [PubMed: 12736377]
38. Henson PM, Bratton DL, Fadok VA. The phosphatidylserine receptor: a crucial molecular switch? *Nat Rev Mol Cell Biol* 2001;2:627–633. [PubMed: 11483996]
39. Bannerman DD, Goldblum SE. Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L899–L914. [PubMed: 12736186]
40. Husain KD, Coopersmith CM. Role of intestinal epithelial apoptosis in survival. *Curr Opin Crit Care* 2003;9:159–163. [PubMed: 12657980]
41. Chung CS, Xu YX, Wang W, Chaudry IH, Ayala A. Is Fas ligand or endotoxin responsible for mucosal lymphocyte apoptosis in sepsis? *Arch Surg* 1998;133:1213–1220. [PubMed: 9820353]
42. Bommhardt U, Chang KC, Swanson PE, et al. Akt decreases lymphocyte apoptosis and improves survival in sepsis. *J Immunol* 2004;172:7583–7591. [PubMed: 15187138]
43. Hotchkiss RS, Tinsley KW, Swanson PE, et al. Prevention of lymphocyte cell death in sepsis improves survival in mice. *Proc Natl Acad Sci USA* 1999;96:14541–14546. [PubMed: 10588741]
44. Hotchkiss RS, Chang KC, Swanson PE, et al. Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. *Nat Immunol* 2000;1:496–501. [PubMed: 11101871]
45. Wesche-Soldato DE, Chung CS, Lomas-Neira J, Doughty LA, Gregory SH, Ayala A. *In vivo* delivery of caspase-8 or Fas siRNA improves the survival of septic mice. *Blood* 2005;106:2295–2301. [PubMed: 15941915]
46. Cauwels A, Janssen B, Waeytens A, Cuvelier C, Brouckaert P. Caspase inhibition causes hyperacute tumor necrosis factor-induced shock via oxidative stress and phospholipase A2. *Nat Immunol* 2003;4:387–393. [PubMed: 12652297]
47. Stephenson DA, Toltl LJ, Beaudin S, Liaw PC. Modulation of monocyte function by activated protein C, a natural anticoagulant. *J Immunol* 2006;177:2115–2122. [PubMed: 16887970]
48. Cheng T, Liu D, Griffin JH, et al. Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med* 2003;9:338–342. [PubMed: 12563316]
49. Andersson J. The inflammatory reflex - introduction. *J Intern Med* 2005;257:122–125. [PubMed: 15656871]
50. Czura CJ, Tracey KJ. Autonomic neural regulation of immunity. *J Intern Med* 2005;257:156–166. [PubMed: 15656874]
51. Ulloa L. The vagus nerve and the nicotinic antiinflammatory pathway. *Nat Rev Drug Discov* 2005;4:673–684. [PubMed: 16056392]
52. Banks WA, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation* 2002;10:319–327. [PubMed: 12907838]
53. Maier SF, Goehler LE, Fleshner M, Watkins LR. The role of the vagus nerve in cytokine-to-brain communication. *Ann NY Acad Sci* 1998;840:289–300. [PubMed: 9629257]

54. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000;405:458–462. [PubMed: 10839541]
55. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;421:384–388. [PubMed: 12508119]
56. Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med* 2004;10:1216–1221. [PubMed: 15502843]
57. Saeed RW, Varma S, Peng-Nemeroff T, et al. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *J Exp Med* 2005;201:1113–1123. [PubMed: 15809354]
58. Borovikova LV, Ivanova S, Nardi D, et al. Role of vagus nerve signaling in CNI-1493-mediated suppression of acute inflammation. *Auton Neurosci* 2000;85:141–147. [PubMed: 11189021]
59. Huston JM, Ochani M, Rosas-Ballina M, et al. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 2006;203:1623–1628. [PubMed: 16785311]
60. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001;345:1368–1377. [PubMed: 11794169]
61. Otero RM, Nguyen HB, Huang DT, et al. Early goal-directed therapy in severe sepsis and septic shock revisited: concepts, controversies, and contemporary findings. *Chest* 2006;130:1579–1595. [PubMed: 17099041]