Inflammation and Coagulation: Implications for the Septic Patient

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Sepsis with acute organ dysfunction (severe sepsis) is common, frequently fatal, and associated with a significant national health/economic burden. In addition to standard care, investigators have focused on interrupting the inflammatory and anti-inflammatory cascade associated with this disease. Unfortunately, despite promising preclinical results, interventions directed at the inflammatory elements have not reduced the morbidity and mortality associated with this disease. Inflammation and coagulation are tightly linked. In fact, sepsis-associated coagulopathy is almost universal in patients with severe sepsis. Preclinical observations indicate that antithrombotic-targeted therapy has the potential to reduce morbidity and mortality in patients with this disease. Treatment with recombinant human activated protein C (drotrecogin α [activated]) was the first antithrombotic-targeted therapy to significantly reduce 28-day all-cause mortality in patients with severe sepsis. The pathophysiological and clinical significance of this evidence and the relationship of coagulation to inflammation are discussed, as are positive and negative results of clinical trials of antithrombotic therapy.

Once the proinflammatory cascade was identified as an important cause of morbidity and mortality in patients with sepsis, investigators targeted the cytokine cascade for innovative antisepsis interventions. Focus on the inflammatory component of the host response has continued despite identification of a significant compensatory anti-inflammatory response syndrome (CARS) that can also increase mortality [1]. Attempts were made to integrate this new information and to characterize patients who would be more likely to benefit from anti-inflammatory therapy. Therefore, investigators developed strategies to identify patients with severe sepsis and septic shock who were still in a proinflammatory state. These included enrolling patients soon after the inflammatory process was identified and targeting patients with increased circulating levels of the proinflammatory cytokine, IL-6. In addition, investigators appreciated that targeting inflammatory components might actually be harmful [2].

More recently, investigators recognized the intimate relationship between procoagulant and proinflammatory activity and hypothesized that antithrombotic strategies might also offer an approach to reduce sepsis-associated mortality [3–6]. Because this relationship is integral to the pathophysiology of sepsis, it has led to preclinical and clinical studies that target both procoagulant and proinflammatory portions of the host response to infection [7]. Here, I describe the inflammatory and counterinflammatory elements of the host’s response to sepsis and discusses unsuccessful attempts to apply anti-inflammatory interventions to patients with severe sepsis. I also discuss the tight linkage between inflammation and the host’s hemostatic response. Finally, I describe the results of clinical trials of antithrombotic interventions.

INFLAMMATION AND SEPSIS

As has been demonstrated in animal models of sepsis, inflammation, tissue injury, and organ dysfunction are
driven by a cascade of proinflammatory cytokines, such as TNF-α and IL-1 [8–10]. In patients with severe sepsis, host response can be characterized as an excessive proinflammatory reaction. Resulting high levels of circulating cytokines can potentiate organ damage by endothelial injury and a multitude of other routes. Endothelial damage is associated with activation of neutrophils and expression of neutrophil and endothelial adhesion molecules (e.g., E- and P-selectins and intercellular adhesion molecule-1). These molecules help localize WBCs to the area of injury; however, before leaving the vessel, inflammatory molecules released by activated neutrophils can produce additional endothelial injury. Release of endothelial cell molecules, such as IL-8 and platelet-activating factor (PAF), attract and activate other cells, amplifying the inflammatory response. This cycle results in focal and ongoing microvascular injury. Other inflammatory factors (e.g., eicosanoids) activate circulating neutrophils; complement receptors on these activated inflammatory cells increase neutrophil adhesion to the endothelium. These observations provide a strong theoretical basis for the role of anti-inflammatory strategies in patients with severe sepsis and septic shock.

CARS IN SEPSIS

Interventions for sepsis that focus on the inflammatory component of the host response ignore the body’s CARS [1]. The CARS includes mediator-neutralizing molecules, such as soluble cytokine receptor and cytokine receptor antagonist. These molecules counteract the excessive release of proinflammatory cytokines by binding to cytokines and interfering with interactions between molecules and membrane-bound receptors. Neutralizing molecules produced by recombinant technology might be used to downregulate the effects of CARS, potentially reducing severe sepsis-associated morbidity and mortality. Decreased expression of human leukocyte antigens (HLA-DR) by monocytes obtained from patients with severe sepsis and CARS indicates a state of immunosuppression [1]. Therefore, patients whose monocytes show decreased HLA-DR expression might benefit from an immunomodulator. This concept was evaluated in severely septic [11] patients treated with filgrastim (G-CSF). Although HLA-DR studies were not performed, administration of G-CSF as an immunostimulant was not associated with differences in morbidity or mortality. Although there are also potential benefits to the suppression of the proinflammatory response, some investigators believe that, if anti-inflammatory molecules are present in excessive amounts, as in CARS, the proinflammatory state is already neutralized. Therefore, therapy targeting the proinflammatory state might produce deleterious immunosuppression [1].

CLINICAL TRIALS OF ANTI-INFLAMMATORY THERAPY IN PATIENTS WITH SEVERE SEPSIS

Strategies have been tested to counteract the runaway proinflammatory state seen in sepsis. These strategies targeted endotoxin and mediators, such as TNF-α, IL-1, PAF, and bradykinin. Other investigational strategies have focused on corticosteroid administration, prostaglandin inhibition, nitric oxide inhibition, and immunoglobulin therapy. Hemofiltration studies have also evaluated the efficacy of cytokine removal as a therapeutic intervention in severe sepsis.

ENDOTOXIN INHIBITION

Endotoxin interacts with cells through specific membrane or soluble receptors. Binding of endotoxin to receptors, such as membrane CD14 or Toll-like receptors, on monocytes and macrophages results in the production of numerous proinflammatory cytokines (e.g., TNF-α, IL-1, and IL-6) and anti-inflammatory cytokines (e.g., IL-10 and IL-12). Furthermore, the interaction upregulates expression of cell adhesion molecules, inducible nitric oxide synthase, phospholipase A2, and inducible cyclooxygenase. Therefore, there is a strong theoretical basis for administration of endotoxin antagonists to patients with severe sepsis of gram-negative bacterial origin. Antiendotoxin strategies have included murine monoclonal antibody (E-5) and mouse-human hybrid monoclonal antibody (HA-1A) against endotoxin. Angus et al. [12] found that, despite adequate sample size and patient enrollment, treatment with E-5 did not improve short-term survival. Similarly, McCloskey et al. [13] found that treatment with HA-1A was not effective at reducing 14-day mortality in patients with bacteremia and septic shock due to gram-negative organisms.

TNF-α INHIBITION

TNF-α is a central proinflammatory cytokine. Anti-TNF-α strategies include monoclonal antibodies against TNF-α and use of neutralizing soluble TNF receptors [2, 14–16]. Monoclonal antibody against TNF-α failed to show statistically significant benefit, as did the P-55 construct soluble TNF-α receptor (type 1). The P-75 (type 2) soluble TNF receptor produced deleterious effects, as did nonspecific blocking of nitric oxide synthase [2, 17].

IL-1 INHIBITION

IL-1 is another proinflammatory molecule that is central to the host’s response to infection. The anti–IL-1 approach was tested with IL-1ra. A 72-h, continuous intravenous infusion failed to result in a statistically significant reduction in mortality [18].
CORTICOSTEROIDS

Corticosteroids downregulate inflammation through a variety of processes. These include inhibition of inducible nitric oxide synthase and downregulation of nuclear factor-κB (NF-κB) with reduced transcription of proinflammatory cytokines and increased transcription of IL-1ra [19]. Although early trials of corticosteroids failed to show benefit [20, 21], a recent 300-patient study by Annane et al. [22] found that patients with septic shock and relative adrenal insufficiency (defined as an increase in the serum cortisol level of <10 μg/dL after a high-dose adrenocorticotropic hormone stimulation test) had a significantly reduced risk of death after treatment with low doses of hydrocortisone and fludrocortisone. These results are encouraging and, one hopes, will be confirmed in larger trials [23, 24].

OTHER APPROACHES

NF-κB is a key intracellular mediator that is responsible for generation of proinflammatory cytokines [25, 26]. After stimulation of various cell receptors with endotoxin, exotoxin, TNF-α, IL-1 oxygen radicals, et cetera, a cytoplasmic kinase is activated, which cleaves the NF-κB inhibitor (IκB) from NF-κB through proteolysis. NF-κB is then translocated to the nucleus and enables the translation, transcription, and production of mRNA targeted for production of a variety of proinflammatory cytokines. Last, anti-inflammatory interventions, such as hemofiltration, immunoglobulin therapy, PAF receptor antagonist, or bradykinin antagonists, have not produced significant benefits or have only been evaluated in small trials [27–30].

COAGULATION AND SEPSIS

There is no debate that inflammation is a major component of sepsis. In 1992, Bone et al. [31] characterized this inflammatory element as the systemic inflammatory response syndrome (SIRS). The authors noted 2 important points: SIRS can be seen in patients with a noninfectious cause, and future work on the inflammatory response to infection would shed light on additional cellular and immunologic mechanisms of sepsis. Since that time, additional work on inflammation has lead to the discovery of the importance of coagulation in sepsis [7, 32–35].

Events that lead to SIRS and eventually the activation of the coagulation cascade can be caused by a myriad of noninfectious insults, including thermal injury, pancreatitis, and trauma [36–38]. Coagulation activation in patients without infection can be indirectly linked to inflammation through thrombin production, TNF-α release, and activation of the complement system [39, 40]. When the proinflammatory response to non-infectious insults is overzealous, activation of the coagulation cascade can occur.

In sepsis, toxins cause direct activation of coagulation via the effect of chemical mediators on the endothelium and monocytes as well as indirect activation through the proinflammatory cascade. Activation of coagulation by toxins occurs directly through upregulation of tissue factor (TF) [35]. TF then activates factor VII of the extrinsic system, leading to thrombin formation and generation of fibrin clots (figure 1). Thrombin production is amplified through the intrinsic system when factor VIIa of the extrinsic system activates factor IX. Thrombin potentiates the prothrombic state when it activates thrombin-activatable fibrinolysis inhibitor (TAFI) [41, 42]. Therefore, thrombin not only produces clots, but also inhibits their removal. In addition, activation of coagulation in sepsis can occur indirectly through the activation of the contact system and release of the coagulation inducers TNF-α and interleukins; bacterial products, such as lipid A (gram-negative product) and

Figure 1. The coagulation cascade. The coagulation cascade is broken down into 2 parts: the intrinsic and extrinsic systems. The primary initiating pathway is the extrinsic system, which is amplified by the intrinsic system. The primary initiator of the extrinsic cascade is tissue factor, which is only exposed after tissue injury. The outcome of the cascade is the polymerization of fibrin monomers into a clot. Adapted from [45, 62].
lipoteichoic acids (gram-positive product), can each cause their release [35, 43, 44].

Sepsis-induced procoagulant activity is generally more severe than that which can be produced by trauma. The prothrombic diathesis is systemic—that is, thrombin is generated not only by the endothelium, but also by circulating activated monocytes. This creates an essentially unlimited supply of TF. Generalized activation of coagulation depletes the body’s natural antithrombotic factors: protein C, antithrombin (AT), and TF pathway inhibitor (TFPI) [45, 46]. Activated protein C is a serine protease that inhibits clotting factors Va and VIIIa. AT is a glycoprotein that inactivates most serine proteases and thrombin. In addition, TFPI inhibits the activity of the VIIa/TF complex of the extrinsic system. By inactivating these factors, natural antithrombotics dampen coagulation. Generation of thrombin also initiates fibrinolysis, primarily through the release of tissue plasminogen activator. Once plasmin is activated, it degrades fibrin, producing fibrin degradation/split products, including D-dimers [47].

The acute respiratory distress syndrome (ARDS) is a common accompaniment of severe sepsis and illustrates the role of prothrombic changes in organ injury/dysfunction [48]. In ARDS, proinflammatory cytokines activate endothelial cells, which leads to activation and accumulation of neutrophils in the pulmonary vasculature. Endothelial cells damaged by inflammatory cytokines and activated neutrophils lose their ability to regulate vascular permeability and become prothrombic. This results in an increase in interstitial water, pulmonary edema, and deposition of intravascular and intraalveolar fibrin, which enhances the inflammatory reaction. Bronchoalveolar lavage fluid from acute lung injury and ARDS reveal increased levels of soluble TF and increased expression of TF by macrophages [49, 50]. Levels of plasminogen activator inhibitor–I are also increased in bronchoalveolar lavage fluid during ARDS [51].

Not only is severe sepsis associated with activation of both the coagulation and inflammatory cascades, but these 2 systems also function in a positive feedback loop [52, 53]. Therefore, a vicious cycle of inflammation and coagulation can result, which has the potential to lead to progressive organ dysfunction and death. Therefore, the hemostatic system is an appropriate target for intervention to reduce morbidity and mortality in patients with severe sepsis.

**CLINICAL TRIALS OF ANTITHROMBOTIC THERAPY IN PATIENTS WITH SEVERE SEPSIS**

A new understanding of sepsis pathophysiology incorporates coagulation and fibrinolysis. Fourrier et al. [54] identified consumptive coagulopathy as a strong predictor of death and multiple-organ dysfunction in patients with septic shock. In that study, the mortality rate was 32% in patients without disseminated intravascular coagulation (n = 16), and it was 77% in patients with disseminated intravascular coagulation (n = 44). In addition, subclinical consumptive coagulopathy can be demonstrated by increased D-dimers in almost all patients with severe sepsis, with a decrease in the protein C level in >80% [55]. Therefore, a coagulopathy of sepsis can be seen in essentially all patients with severe sepsis. The earliest signs of consumptive coagulopathy in sepsis are a decrease in protein C and an increase in D-dimer. In patients with more-severe consumptive coagulopathy, prothrombin time and partial thromboplastin time increase, with a decrease in the fibrinogen level and platelet count. Fibrinolysis is also impaired in severe sepsis. Thus, the net result is a proinflammatory and prothrombic disorder with impaired fibrinolysis. This pathophysiologic triad is an attractive target for antisepsis interventions.

Two approaches have potential benefits in patients with sepsis-associated coagulopathy: (1) administration of TFPI or a VIIa/TF inhibitor to block TF-mediated activation of the extrinsic system, and (2) restoration of endogenous natural antithrombotic mechanisms through administration of AT concentrate or activated protein C. All of these compounds would be expected to have both antithrombotic and anti-inflammatory effects.

**RECOMBINANT HUMAN TFPI**

After stimulation by bacterial products, monocytes and perhaps a subset of endothelial cells upregulate expression of TF [56, 57]. Extrinsic pathway activation is tightly regulated by TFPI, a potent inhibitor of TF. Other inhibitors of the VIIa/TF complex include antifactor VII monoclonal antibody and inactivated factor VIIa (DEGR-FVIIa), a form of factor VIIa with its active center blocked, preventing interaction of factor VII with TF. In addition, low-molecular-weight inhibitors, such as the synthetic protease inhibitor nafamostat, have also been tested in sepsis models [58].

After successful preclinical trials, a phase 2 study of TFPI revealed promising data as to the potential for clinical outcome benefit [59]. Unfortunately, results of a large phase 3 trial of TFPI in patients with severe sepsis completed in late 2001 failed to demonstrate a mortality benefit for recombinant TFPI [60].

**RECOMBINANT HUMAN AT**

AT is a serine protease inhibitor of hepatic origin [61]. It inhibits multiple components of the intrinsic, extrinsic and final common coagulation pathways (IIa, IXa, Xa, Xla, XIIa, VIIa/TF, and kallikrein) [45, 62]. In addition to its antithrombotic properties, AT bound to endothelial cell surface heparans stimulates the endothelium to release prostacyclin, a molecule with
anti-inflammatory properties. In animal models, AT successfully blocks endotoxin-induced pulmonary leukocyte accumulation and vascular permeability changes [63]. Despite these encouraging preclinical data, a large \((n = 2314)\) phase 3 clinical trial of AT in patients with severe sepsis failed to show overall benefit [64]. A subgroup analysis suggested that patients who were receiving concomitant heparin had significantly increased bleeding, whereas a trend in improved 28-day survival, which became significant at 90 days, was demonstrated in a subgroup analysis of patients not receiving heparin. This suggests, perhaps, that binding of the natural and therapeutic AT prevented the recombinant AT from exerting an anti-inflammatory effect.

**RECOMBINANT HUMAN ACTIVATED PROTEIN C**

Protein C is converted to activated protein C by thrombin bound to thrombomodulin—a reaction that is facilitated by the endothelial protein C receptor. In patients with severe sepsis, conversion of protein C to its activated form is impaired, resulting from endothelial cell dysfunction/damage. Exogenous administration of protein C is ineffective in raising activated protein C levels in the presence of significant endothelial damage [65]. Therefore, activated protein C must be available to counter the sepsis-induced prothrombic state. Activated protein C has also been demonstrated in vitro to have significant anti-inflammatory properties associated with a decrease in pro-inflammatory cytokines and a reduction of endothelial neutrophil rolling. Activated protein C also inactivates factors Va and VIIIa, downregulating thrombin formation and thereby indirectly preventing the activation of TAFI [66]. Activated protein C also inactivates plasminogen activator inhibitor—1 by forming a complex with the latter [67]. Experimentally, recombinant human activated protein C also inhibits endothelial cell apoptosis [68]. Therefore, this molecule demonstrates many desirable characteristics of potential clinical utility in patients with severe sepsis and septic shock.

On the basis of encouraging results from a phase 2 trial of recombinant human activated protein C (drotrecogin \(\alpha\) [activated]), an international phase 3 trial of activated protein C was conducted [69]. This randomized, double-blind, placebo-controlled, multicenter trial enrolled patients with severe sepsis. Most patients were undergoing mechanical ventilation and required vasopressors. A dosage of 24 \(\mu g/kg/h\) of drotrecogin \(\alpha\) (activated) was administered for a total duration of 96 h. The primary efficacy end point was 28-day all-cause mortality.

The trial was stopped at the time of the second interim analysis by an independent data and safety monitoring board because of overwhelming efficacy that met predefined stopping rules. At that time, 1728 patients were enrolled in the trial. A 6.1% absolute and a 19.4% relative risk reduction in mortality was associated with receipt of drotrecogin \(\alpha\) (activated) treatment \((P = .005)\) [69]. The treatment effect was primarily in the third and fourth APACHE II quartiles, which demonstrated an absolute risk reduction of 13.0% and a relative risk reduction of 29.0% [70].

There was a non–statistically significant increase in cases of severe bleeding in the drotrecogin \(\alpha\) (activated) group \((P = .06)\); therefore, increased risk of severe bleeding with use of this drug should be assumed. Bleeding occurred primarily during the drug infusion and was procedure related. Although there is an increased risk of bleeding, the population as a whole, regardless of the relationship placed on risk of serious bleeding versus benefit of reduced mortality, demonstrated a good benefit-to-risk profile associated with administration of drotrecogin \(\alpha\) (activated) [69, 71].

Phase 3 trial data indicated that 1 additional life would be saved for every 16 patients treated with drotrecogin \(\alpha\) (activated) and 1 life would be saved per 8 patients treated in the third and fourth APACHE II quartiles (Eli Lilly, data on file) [70]. In fact, it is not unreasonable to compare drotrecogin \(\alpha\) (activated) with tissue plasminogen activator therapy for acute myocardial infarction [72]. Both therapies save lives. Both therapies are associated with the possibility of serious bleeding, including intracranial hemorrhage. Both drugs are associated with significant expense. With both drugs, the risk of bleeding and the expense are well worth the potential benefit, if proper care and deliberation are provided before deciding in favor of administration.

**POSITIVE AND NEGATIVE TRIALS WITH ANTITHROMBOTIC THERAPY**

The explanations for the success of drotrecogin \(\alpha\) (activated) and the failures of both TFPI and AT are not entirely clear. The difference may be related to trial design issues, to dosing differences, or, more likely, to inherent differences in the anticoagulant and anti-inflammatory properties of the molecules.

**CONCLUSION**

During the past decade, considerable advances have been made in the knowledge of the pathophysiology of severe sepsis. In addition, randomized clinical trials of critical care interventions, such as low tidal volume ventilation and glucose control, have reduced mortality more effectively than conventional therapy [73, 74]. Advances in supportive care have not been paralleled by success with antisepsis interventions. Many trials of agents solely targeting the inflammatory cascade have failed to reduce 28-day all-cause mortality. With the knowledge that the innate immune system is closely linked to the coagulation cascade, the antithrombotic approach now offers great promise.
for bedside application of antisepsis therapy that will improve survival.

References