Selected Treatment Strategies for Septic Shock Based on Proposed Mechanisms of Pathogenesis

Moderator: Charles Natanson, MD; Discussants: William D. Hoffman, MD; Anthony F. Suffredini, MD; Peter Q. Eichacker, MD; and Robert L. Danner, MD

**Purpose:** To review selected new therapies for septic shock designed to inhibit bacterial toxins or endogenous mediators of inflammation.

**Data Sources:** Scientific journals, scientific meeting proceedings, and Food and Drug Administration advisory committee proceedings.

**Study Selection and Extraction:** Preclinical and clinical data from trials using core-directed antiendotoxin antibodies and anticytokine therapies for sepsis and studies in animal models of sepsis from our laboratory.

**Results of Data Synthesis:** Ten clinical trials using core-directed antiendotoxin antibodies produced inconsistent results and did not conclusively establish the safety or benefit of this approach. Both anti-interleukin-1 and anti-tumor necrosis factor (TNF) therapies have been beneficial in some animal models of sepsis but did not clearly improve survival in initial human trials, and one anti-TNF therapy actually produced harm. Neutrophils, another target for therapeutic intervention, protect the host from infection but may also contribute to the development of tissue injury during sepsis. In a canine model of septic shock, granulocyte colony-stimulating factor increased the number of circulating neutrophils and improved survival, but an anti-integrin (CD11/18) antibody that inhibits neutrophil function worsened outcome. Nitric oxide, a vasodilator produced by the host, causes hypotension during septic shock but may also protect the endothelium and maintain organ blood flow. In dogs challenged with endotoxin, the inhibition of nitric oxide production decreased cardiac index and did not improve survival.

**Conclusions:** No new therapy for sepsis has shown clinical efficacy. Perhaps more accurate clinical and laboratory predictors are needed to identify patients who may benefit from a given treatment strategy. On the other hand, the therapeutic premises may be flawed. Targeting a single microbial toxin such as endotoxin may not represent a viable strategy for treating a complex inflammatory response to diverse gram-negative bacteria. Similarly, the strategy of inhibiting the host inflammatory response may not be beneficial because immune cells and cytokines play both pathogenic and protective roles. Finally, our scientific knowledge of the complex timing of mediator release and balance during sepsis may be insufficient to develop successful therapeutic interventions for this syndrome.

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Dr. Charles Natanson (Critical Care Medicine Department, Clinical Center, National Institutes of Health [NIH], Bethesda, Maryland): Sepsis and septic shock are heterogeneous clinical syndromes that can be triggered by many microorganisms, including gram-negative bacteria, gram-positive bacteria, and fungi (1-3). Participants in a recent consensus conference tried to define sepsis and septic shock. Sepsis was characterized as a systemic response to infection manifested by tachycardia, tachypnea, change in temperature, and leukopenia or leukocytosis. Septic shock was defined as severe sepsis accompanied by hypotension (4). However, patients with sepsis may have one or several signs and symptoms, and no single physiologic or laboratory parameter can universally identify this syndrome.

Advances in molecular biology and immunology during the past decade have increased our understanding of the pathogenesis of septic shock (Figure 1). In particular, we now believe that the host's inflammatory response to infection contributes substantially to the development of septic shock (5-7). Infections begin when microorganisms circumvent or penetrate host barriers such as skin and mucosa. Depending on the infecting agent's virulence and the patient's immunocompetence, local host defenses may be overwhelmed, resulting in microbial invasion of the bloodstream. Toxic bacterial products present in the circulation activate systemic host defenses, including plasma factors (complement and clotting cascades) and cellular components (neutrophils, monocytes, macrophages, and endothelial cells). In turn, activated cells produce potentially toxic host mediators (cytokines such as tumor necrosis factor [TNF] and interleukin-1 [IL-1], kinins, eicosinoids, platelet-activating factor, and nitric oxide) that augment the inflammatory response. This escalating immune response, in concert with microbial toxins, can lead to shock, multiple organ failure, and death.

Standard sepsis treatment strategies include use of antibiotics to kill invading bacteria, surgical procedures to eradicate the nidus of infection, and intensive life-support procedures such as dialysis, mechanical venti-
Pathogenesis of Septic Shock

Figure 1. The pathogenesis and treatment of septic shock. Solid black arrows follow the pathogenesis of septic shock beginning with a nidus of infection and ending in shock and multiple organ failure. Open arrows indicate treatment strategies.

Antendotoxin Therapies in Septic Shock

Dr. William D. Hoffman (Critical Care Medicine Department, Clinical Center, NIH, Bethesda, Maryland): The outer membrane of gram-negative bacteria contains lipopolysaccharides called endotoxin (14). Endotoxin induces an inflammatory response that may protect the host from infection but may also cause multiple-organ failure and death when present in excess amounts. Specific immunochemical properties have been associated with different components of the endotoxin molecule. The O-polysaccharide chain (O-side chain) of endotoxin is exposed on the outside surface of gram-negative bacteria. The O-side chain is not toxic when injected into animals and has a molecular structure that varies among gram-negative bacteria. The core sugar and lipid A regions of endotoxin are embedded deeply in the outer bacterial membrane, and their molecular structures are similar for all gram-negative bacteria. In contrast to the O-side chain, lipid A is toxic when given to animals (14).

Effects of Endotoxin Challenge—Endotoxemia in Sepsis

Experimental observations have supported and challenged the concept that endotoxin-directed therapies can benefit patients with septic shock. Reversible organ dysfunction and hemodynamic changes that are qualitatively similar to those seen in patients with septic shock develop in animals injected with endotoxin (15) and healthy human volunteers injected with safe doses of endotoxin (16). In addition, development of endotoxemia in patients with septic shock has been associated with severe organ damage (9). However, neither induced tolerance to endotoxin in humans (17) nor genetic resistance to endotoxin in mice (18) is protective during gram-negative infections. In addition, increased sensitivity to endotoxin does not alter the course of gram-negative infection in animals (19). Finally, endotoxin and endotoxemia are not necessary to produce the septic shock syndrome, and endotoxin may be only one of many bacterial products that can trigger the septic response (3, 15).

Approaches to Antendotoxin Therapy

Although no antiendotoxin therapy is in clinical use, several are being investigated (Table 1). Antibodies to the O-side chain produce serotype-specific (20), complement-dependent bactericidal activity (21). However, serotype specificity limits the clinical utility of O-side chain therapies because treating patients empirically with an effective dose of antibody for every probable infecting bacterial strain would be difficult. This problem led to investigation of antibodies directed at core and lipid A structures of endotoxin, because these antibodies might cross-protect against diverse gram-negative bacteria (22). Although core or lipid A antibodies were thought to mediate antiendotoxin (23) or endotoxin-clearing effects (24), the function of these antibodies is unknown and controversial (25–28). Nevertheless, core-directed antibodies are the only antiendotoxin therapies studied in clinical trials. Other antiendotoxin agents listed in Table 1 may reduce the host inflammatory response by directly neutralizing endotoxin, increasing its clearance, antagonizing its effects on host cells, or inducing tolerance. Controlled therapeutic trials of agents that reduce the bioactivity of endotoxin and have no antibacterial effect may determine whether circulating endotoxin is a useful therapeutic target in septic shock.

Polyclonal Antibodies Directed at Core Epitopes and Lipid A

The first clinical trial of core-directed antibodies studied patients with gram-negative bacteremia treated with...
<table>
<thead>
<tr>
<th>Agent</th>
<th>Possible Effect</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS O-side chain-directed antibody</td>
<td>Complement-dependent bactericidal activity against specific serotype of gram-negative organism</td>
<td>Octavalent <em>Pseudomonas aeruginosa</em> vaccine</td>
</tr>
<tr>
<td>LPS core- or lipid A-directed antibody</td>
<td>May enhance endotoxin clearance in many gram-negative infections</td>
<td>HA-1A, E5, J5 immune plasma or serum†</td>
</tr>
<tr>
<td>Peptides and lipoproteins that bind endotoxin</td>
<td>Neutralize endotoxin</td>
<td>Cationic polypeptides (polymyxin B, colistin)</td>
</tr>
<tr>
<td></td>
<td>Enhance clearance of endotoxin</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>Lipid A derivatives</td>
<td>Induce tolerance to endotoxin</td>
<td>Desacylated endotoxins</td>
</tr>
<tr>
<td></td>
<td>Direct antagonism of endotoxin</td>
<td>Lipid X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monoacylphosphorylated lipid A</td>
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* LPS = lipopolysaccharide.
† Clinical trials have been performed using these agents.

control (n = 100) or J5 antiserum (n = 91) (8). In that study, 21 of 39 patients with localized gram-negative infection but no bacteremia were included in the gram-negative bacteremia group because they had been given appropriate antibiotics before blood cultures were obtained (8). The sepsis-related mortality rate for patients with gram-negative bacteremia given J5 antiserum was 22% (compared with 39% with control serum). In a subgroup of patients who required vasopressor drugs for more than 6 hours, the mortality rate was 44% (compared with 77% with control serum). The effect of J5 antiserum on mortality from all causes or in patients with gram-negative infection was not reported (8). Five subsequent clinical trials (Table 2) using polyclonal core-reactive antiserum or immunoglobulin to prevent or treat gram-negative sepsis showed essentially no survival benefit (29–34).

Monoclonal Antibodies Directed at Core Epitopes and Lipid A: E5 and HA-1A

Monoclonal antibodies were developed to produce a more specific antiendotoxin therapy with less risk for transmission of infection. E5, a murine IgM, protected mice injected with bacteria (35), and HA-1A, a human IgM, protected mice and rabbits injected with bacteria (36). However, E5 did not protect sheep given endotoxin (37), and other researchers subsequently did not reproduce the beneficial effects of HA-1A in mice and rabbits (38).

**E5 Clinical Trials**

E5 was tested in two multicenter, randomized, placebo-controlled clinical trials (Table 2). In the first trial of 468 patients, E5 provided no significant benefit to patients with gram-negative infection. The antibody improved survival in a retrospectively identified subgroup of 137 patients with gram-negative infection without refractory shock (30% compared with 43%; P = 0.01) (39). A second trial of 847 patients was conducted to confirm this favorable effect (Table 2). However, in the second study, E5 did not significantly improve survival in the 530 patients who had gram-negative infection without refractory shock (E5, 30% mortality compared with control, 26%; P = 0.21) (40). Using a meta-analysis and combining data from the two trials, researchers found that E5 substantially decreased the time to recovery from organ dysfunction and improved survival in a subgroup of patients with gram-negative infection and organ dysfunction who were not in refractory shock (unpublished data). A third multicenter clinical trial of E5 is being done.

**HA-1A Clinical Trials**

In a randomized clinical trial of 543 patients, HA-1A also failed to increase survival in patients with documented gram-negative infection (10) (Table 2). The authors reported that HA-1A significantly decreased mortality from all causes at 28 days in a subgroup of patients with gram-negative bacteremia (30% compared with 49%; P = 0.014). Based primarily on this study, HA-1A was approved for clinical use in Europe. However, the validity of these results was questioned, because the published report did not document (41–44) the multiple end points and subgroups analyzed and therefore did not appropriately adjust probability values (42). Furthermore, results were available to staff at Centocor (the manufacturer of HA-1A and a collaborator in the clinical trial) before the clinical trial was completed. The analytic plan was changed after Centocor learned these interim results, which introduced the potential for bias in these findings (43, 44). When the data were analyzed using the original analytic plan, HA-1A did not show a significant effect on survival (P = 0.12) (41–44).

In our canine model of septic shock, a murine IgG monoclonal antibody specific for core structures of endotoxin improved hemodynamics and produced a nonsignificant trend toward prolonged survival (45). Encouraged by these results, we performed a blinded, controlled clinical trial of HA-1A in dogs with septic shock. Unexpectedly, HA-1A significantly decreased 28-day survival when compared with controls (15% compared with 57%; P = 0.034; Figure 2) (46). A more severe septic shock syndrome occurred in dogs given HA-1A, and it had no effect on endotoxemia. In addition, cardiac performance at 24 hours was worse in HA-1A–treated animals and seemed to result...
from greater cardiac diastolic dysfunction. Conceivably, the previously described nonspecific binding of HA-1A to cardiolipin (an antigen in cardiac muscle) (29) could have contributed to this unanticipated harmful effect.

In April 1992, the Food and Drug Administration decided that a second clinical trial of HA-1A was needed to determine whether the drug was safe and effective in treating gram-negative sepsis. The second HA-1A trial was terminated in January 1993 after an interim monitoring of data revealed a higher mortality rate in patients randomized to receive HA-1A (44) (Table 2). Consequently, the manufacturer removed HA-1A from the European market. Investigators have not reported an explanation for the excess mortality associated with HA-1A.

The Future of Antiendotoxin Therapies

Although these results are discouraging, they do not preclude the possibility that endotoxin is a valuable treatment strategy for septic shock. The antibodies studied in clinical trials did not bind to endotoxin with high affinity and did not neutralize its effects (28, 38). Newer agents that bind to and neutralize endotoxin are being developed, including peptides (nontoxic derivatives of polymyxin B and neutrophil-derived bactericidal/permeability-increasing protein) and lipoproteins (high-density lipoproteins) (47) (see Table 1). In addition, investigators are examining several lipid A derivatives that may diminish toxicity. These compounds either directly antagonize endotoxin or simulate its beneficial immunostimulatory properties, such as induction of tolerance and increased nonspecific resistance to infection. Clinical trials using these antiendotoxin agents are being planned.

In summary, antiendotoxin core-directed antibodies have not shown an important survival benefit in 10 clinical trials (see Table 2), and the cross-protection hypothesis (22) remains unproved. Whether endotoxin is an appropriate therapeutic target for the treatment of septic shock is still unclear.

Anticytokine Therapies

Dr. Anthony F. Suffredini (Critical Care Medicine Department, Clinical Center, NIH, Bethesda, Maryland): Cytokines are peptides that function as cellular signals to regulate the amplitude and duration of the host’s inflammatory response (6). The roles of two cytokines, TNF and IL-1, in septic shock have been studied extensively. Monocytes release TNF and IL-1 when exposed in vitro to bacterial components such as endotoxin and can be detected in the blood of humans and animals during bacterial infection or after administration of endotoxin (48–50). When administered to animals or humans, recombinant IL-1 and TNF reproduce many of the manifestations of septic shock (51–54). Conversely,

Table 2. Summary of 10 Clinical Trials with Lipopolysaccharide Core-directed Antibodies*

<table>
<thead>
<tr>
<th>Therapy (Reference)</th>
<th>Targeted Patient Group (Prospective Analysis)</th>
<th>Mortality Rate, n/n (%)</th>
<th>P Value for Mortality</th>
<th>Reported Subgroups</th>
<th>P Value for Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>J5 antiserum (8)</td>
<td>Gram-negative bacteremia†</td>
<td>23/103 (22)</td>
<td>0.011</td>
<td></td>
<td></td>
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<tr>
<td>J5 antiserum (31)</td>
<td>Prophylaxis in neutropenic patients</td>
<td>4/47 (9)</td>
<td>0.41</td>
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<tr>
<td>J5 immune plasma</td>
<td>Prophylaxis in high-risk surgical patients</td>
<td>14/126 (11)</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J5 immune plasma</td>
<td>Infectious purpura in children</td>
<td>10/40 (25)</td>
<td>0.31§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J5 intravenous</td>
<td>Gram-negative septic shock</td>
<td>15/30 (50)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E5 (39)</td>
<td>Gram-negative sepsis (30-day mortality)</td>
<td>63/164 (38)</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E5 (40)</td>
<td>Gram-negative sepsis nonrefractory shock</td>
<td>79/264 (30)</td>
<td>0.21**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA-1A (10)</td>
<td>Gram-negative bacteremia (14-day mortality)</td>
<td>25/105 (24)</td>
<td>0.12**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA-1A (44)</td>
<td>Gram-negative bacteremia (14-day mortality)</td>
<td>Early stopping criteria were met for excess mortality in the HA-1A group</td>
<td></td>
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</table>

* LPS = lipopolysaccharide.
† Included patients with gram-negative infection and negative results of blood cultures when taking appropriate antibiotics.
‡ One-tailed test. Two-tailed test, P = 0.06.
§ Imbalances in risk factors negated this trend toward a beneficial effect.
¶ Not the primary end point in analytic plan.
|| Fisher exact test calculated from reported survival rates.
** As reported at a Food and Drug Administration Advisory Meeting, 4 September 1991.
Variability among studies in the reported levels and the blood of septic patients, but levels vary considerably. Tumor necrosis factor and IL-1 are detected in the absence of measurable amounts in the circulation (59). Bronchoalveolar lavage obtained from septic patients contained markedly increased levels of TNF and IL-1, whereas plasma obtained simultaneously from the same patients contained lower levels of these cytokines (61). In contrast, healthy persons given intravenous endotoxin had increased levels of circulating TNF but little or no TNF in bronchoalveolar lavage (62). Thus, circulating cytokines do not always reflect the level of inflammatory response of tissue compartments.

After their release from cells, TNF and IL-1 may act locally within their microenvironment or be released into the circulation, where they may bind to carrier proteins or to their respective extracellular receptors. These receptors are shed by cells, including endothelial cells and neutrophils, during acute inflammation (5, 63). Cytokine binding to these carrier proteins and shed receptors may increase their half-life, change cytokine interactions with cell-associated receptors, and protect the cytokine from degradation by activated proteases (64, 65) (Figure 3). The effects of IL-1 are also modulated by IL-1 receptor antagonist (IL-1ra) (5), an acute-phase cytokine structurally similar to IL-1 that can block the IL-1 receptor site and prevent activation of the target cell by IL-1 (5). Increased concentrations of soluble TNF receptors (60 and 80 kd) and the IL-1 receptor antagonist (IL-1ra) are found in the blood of healthy volunteers challenged with intravenous endotoxin and in patients with acute infection (66, 67). Tumor necrosis factor and IL-1 play protective roles in the immune response to infection. Both recruit and activate neutrophils, macrophages, and lymphocytes and increase gene expression and release of acute-phase proteins and granulocyte colony-stimulating factors (68).

Increased concentrations of TNF protected against lethal challenges of endotoxin, host defenses (69, 70). Similarly, low doses of IL-1 or TNF protected against lethal challenges of endotoxin, TNF, or bacteria in rats and mice (71, 72).

In animal models of infection caused by intracellular pathogens such as Legionella or Candida species, TNF inhibition worsened outcome, presumably by impairing host defenses (69, 70). In addition to exerting beneficial proinflammatory effects on host defense, TNF and IL-1 also mediate anti-inflammatory responses that may protect against tissue injury. These include gene expression for manganese superoxide dismutase and cyclo-oxygenase, release of acute-phase proteins and antiproteases, induction of anti-inflammatory cytokines (for example, IL-4, IL-6, and IL-10; transforming growth factor b; and IL-1ra), and down-regulation of TNF and IL-1 receptors (68). Thus, cytokines play a critical role in regulating host defense during septic shock.

A basic tenet underlying the anticytokine approach to sepsis is that exaggerated inflammatory responses develop in some patients, perhaps because of failed normal inflammation control mechanisms. However, determining when a cytokine-mediated inflammatory event is harmful or beneficial is difficult clinically. Therefore, cytokine suppression may be dangerous during a severe infection. This issue is important when designing anticytokine trials and using these agents therapeutically.

Many studies were published that describe TNF and IL-1 antagonists in animal models of infection, but few clinical trials in humans have been published in peer-reviewed journals, and results from other clinical trials

inhibition of TNF and IL-1 protects animals given endotoxin or bacteria (7, 55–57).

Tumor necrosis factor and IL-1 are detected in the blood of septic patients, but levels vary considerably among studies. Increased levels of TNF are reported more often than IL-1, but both have been detected in patients infected with diverse types of micro-organisms (58, 59). In patients with meningococcemia, circulating TNF correlated directly with death (60). However, in patients with heterogeneous infections complicated by septic shock, IL-1 and TNF levels were no more predictive of clinical outcome than were factors such as age, bacteremia, urine output, and arterial pH (58). Methodologic differences in blood sample timing and in the assays (biologic assay compared with immunoassay) used to measure cytokines may explain, in part, the variability among studies in the reported levels and the prognostic importance of TNF and IL-1.

Circulating levels of a given cytokine, however, may not indicate the magnitude of the host inflammatory response. During sepsis or septic shock, monocyte-associated TNF and IL-1 were documented in the absence of measurable amounts in the circulation (59). Bronchoalveolar lavage obtained from septic patients with adult respiratory distress syndrome contained markedly increased levels of TNF and IL-1, whereas plasma obtained simultaneously from the same patients contained lower levels of these cytokines (61). In contrast, healthy persons given intravenous endotoxin had increased levels of circulating TNF but little or no TNF in bronchoalveolar lavage (62). Thus, circulating cytokines do not always reflect the level of inflammatory response of tissue compartments.

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Studies of IL-1 antagonists have been limited primarily to the use of IL-1ra. Recombinant IL-1ra (Synergen Inc., Boulder, Colorado) improved survival in animal models of sepsis when given before or as long as 3 hours after bacterial challenge (55, 57). A randomized, open-label, phase II trial in 99 patients with sepsis or septic shock showed improved survival at 28 days that corresponded to increasing doses of IL-1ra (placebo: 44%; IL-1ra: 17 mg/kg, 32%; 67 mg/kg, 25%; 133 mg/kg, 16%) (82). In a subsequent randomized, double-blind, phase III multicenter trial of IL-1ra in 893 patients with sepsis or septic shock, no significant difference in 28-day mortality was found among three treatment groups (placebo: 34%; IL-1ra: 1 mg/kg per hour, 31%; 2 mg/kg per hour, 29%; P = 0.22) (83). At study entry, IL-1ra also had no effect in 713 patients with shock (placebo: 36%; IL-1ra: 1 mg/kg per hour, 31%; 2 mg/kg per hour, 31%; P = 0.23). A retrospective analysis was performed based on a risk prediction model for death from sepsis (84). This analysis identified 595 patients with sepsis syndrome (with or without shock) who had a predictive risk for death at study entry of 24% or more. Using this risk prediction model scoring system, IL-1ra decreased 28-day mortality rate in high-risk patients by 18% in low-dose and by 22% in high-dose groups (placebo: 45%; IL-1ra: 1 mg/kg per hour, 37%; 2 mg/kg per hour, 35%) (83). A second clinical trial is being done to test this hypothesis prospectively.

Anti-TNF and anti-IL-1 agents have not been shown to improve outcome in the treatment of human sepsis and septic shock and may, in fact, be potentially harmful. Identification of patients in whom an exaggerated cytokine response develops and who are thus likely to benefit from anti-inflammatory treatment strategies remains an important issue. The timing, duration, and delivery of these therapies to tissue compartments (to the peritoneum or lung, for example) are other critical unresolved issues. Clearly, suppression of cytokine function may be injurious for some patients, and the effect of these agents on bacterial clearance, nosocomial infection, and the reparative processes after tissue damage from sepsis requires further investigation. Whether it is clinically feasible to inhibit cytokines and limit their harmful effects while preserving their ability to perform necessary beneficial functions is unknown. The lack of efficacy in four clinical trials (73, 80, 81, 83) and harm produced by one TNF antagonist (81) prompts questions about the methodology used, the viability of this therapeutic approach, or both.

The Neutrophil as a Therapeutic Target in Septic Shock

Dr. Peter O. Eichacker (Critical Care Medicine Department, Clinical Center, NIH, and Armed Forces Radiobiology Research Institute, Bethesda, Maryland): Activation of the host defense system is needed for microbial clearance during sepsis. However, host inflammatory mediators activated and released in response to infection may contribute to the cascade of harmful clinical events associated with this syndrome (5–7). The function of neutrophils in the development of sepsis illustrates the complex, divergent consequences of the host immune response.

Figure 3. Regulation of cytokine activity in vivo. Reproduced from R. Fernandez-Botran and the American Physiological Society (65) and modified with permission.
Several studies have shown that the neutrophil and its toxic byproducts can produce tissue injury and organ dysfunction in sepsis and septic shock (85–88) and that neutrophil inhibition or depletion improves outcome in some animal models (89). However, the neutrophil is also a key component of host defense (90). Reduced neutrophil count and abnormal function predispose humans to infection (91–93). More importantly, therapies that augment neutrophil count or function reduce the risk for infection in neutropenic animals and humans (94). These data suggest that therapies to inhibit neutrophil activity during sepsis must not interfere with the protective role this cell plays in host defense.

New anti-inflammatory therapies for septic shock that may indirectly impair neutrophil function (for example, IL-1ra and TNF-directed monoclonal antibody) have not shown convincing clinical benefits (73, 83). To investigate further the potential of neutrophil inhibition as a treatment strategy for sepsis, we used a canine model to study the effects of monoclonal antibodies directed against the leukocyte CD11/18 adhesion complex (CD11/18 monoclonal antibodies), a cell-surface receptor that regulates neutrophil-endothelial cell adhesion, the first step in neutrophil migration to sites of infection and inflammation (95). Key inflammatory mediators of sepsis, such as endotoxin, IL-1, and TNF, stimulate neutrophil adherence to endothelium through this complex (96). Monoclonal antibodies against the CD11/18 complex in vitro and in vivo prevent endotoxin, TNF-, and complement-induced neutrophil adhesion and injury to endothelial cells and neutrophil extravascular migration (97–100). Use of such antibodies in different animal models of sepsis by other investigators have had either beneficial or harmful effects (101, 102).

In the first CD11/18 monoclonal antibody study, we pretreated animals with monoclonal antibodies directed against this complex and then challenged them with recombinant TNF (103). In animals, TNF challenge produces dose-dependent lethal effects and cardiopulmonary injury with hypotension, reduced cardiac function (left ventricular function), and hypoxemia similar to that observed with bacterial challenge (104). During the first 24 hours after TNF infusion, CD11/18 monoclonal antibodies reduced mortality rates from 71% to 42% (P < 0.05) and increased arterial oxygenation (P < 0.05) (103). However, these beneficial effects did not persist, and overall survival (more than 7 days) and cardiopulmonary dysfunction in monoclonal antibody- and placebo-treated animals did not differ. These findings suggest that CD11/18 complex-dependent mechanisms are important in the early (at 24 hours) but not late stages of TNF-induced tissue injury.

In the second study, we investigated the effects of CD11/18 monoclonal antibody in dogs challenged with Escherichia coli intraperitoneal clots and treated with antibiotics. Unexpectedly, administration of CD11/18 monoclonal antibodies in animals challenged with bacteria was harmful (105). Four of eight animals treated with CD11/18 monoclonal antibody and two of eight controls died during the study (P = 0.4). However, the study was terminated before this lethal effect reached statistical significance because animals treated with CD11/18 monoclonal antibody had significantly greater decreases (P < 0.05) in mean arterial pressure, cardiac index, central venous pressure, and arterial pH and had larger increases (P < 0.05) in arterial lactate than did control animals.

These findings indicate that CD11/18 monoclonal antibody worsened cardiovascular instability and decreased tissue perfusion during sepsis. Further, compared with controls, animals treated with CD11/18 monoclonal antibody had higher (P < 0.05) serum endotoxin levels as long as 8 hours after clot placement. Together, these data suggest that CD11/18 monoclonal antibody therapy was harmful because it impaired host mechanisms that clear toxic bacterial products such as endotoxin. Increases (P < 0.05) in circulating neutrophils in the CD11/18 monoclonal antibody group suggest that neutrophil-endothelial cell adhesion was inhibited in treated animals.

In a similar experiment, we studied the effects of augmenting neutrophil count and function with recombinant granulocyte colony-stimulating factor (Amgen, Thousand Oaks, California), a potent stimulator in vitro and in vivo of immature and mature neutrophil function (106, 107). Granulocyte colony-stimulating factor therapy previously was shown to have beneficial effects in immunocompetent models of infection (108, 109). We treated animals with granulocyte colony-stimulating factor or placebo for 9 days before and 3 days after bacterial challenge (110). Granulocyte colony-stimulating factor increased peripheral neutrophil counts five to six times (that is, 50 000 to 60 000 cells/mm³) before infection. During septic shock, granulocyte colony-stimulating factor increased circulating neutrophils (P < 0.001) and alveolar neutrophils (P < 0.05) (determined by lavage) but did not affect pulmonary function. In fact, after the onset of sepsis, granulocyte colony-stimulating factor therapy was associated with prolonged survival times (P < 0.04), improved mean arterial blood pressure (P < 0.02), and cardiac function (left ventricular ejection fraction, P < 0.001). In contrast to the decreases in endotoxin clearance from blood with CD11/18 antibody therapy, granulocyte colony-stimulating factor treatment was associated with increases (P < 0.02) in endotoxin clearance. These findings suggest that treatment with granulocyte colony-stimulating factor at doses sufficient to increase the number of circulating neutrophils and to accelerate extravascular neutrophil recruitment was associated with improved clearance of endotoxin and potentially better control of infection in this canine model of sepsis.

Although inhibition of neutrophil function by CD11/18 monoclonal antibodies may acutely blunt the harmful effects of inflammatory mediators such as TNF during bacterial infection, this inhibition may also impair host defense and ultimately lead to more severe tissue injury. Clearly, during sepsis, therapies to limit inflammatory tissue injury must not inhibit necessary neutrophil-related host defenses if they are to produce a net benefit. Neutrophil function augmented with granulocyte colony-stimulating factor during sepsis may benefit some patients by boosting host defenses and by increasing the clearance of microbial toxins.
Nitric Oxide: A Therapeutic Target in Sepsis

Dr. Robert L. Danner (Critical Care Medicine Department, Clinical Center, NIH, Bethesda, Maryland): Nitric oxide is a low-molecular-weight, membrane-permeable gas (111) that functions as a neurotransmitter (112), regulates vascular tone (113), and inhibits platelet aggregation (114) and leukocyte adhesion (115). In addition, at concentrations higher than those required for intercellular communication, nitric oxide has antitumor and antimicrobial activity (116). Inhibition of nitric oxide production has been proposed as a new approach to treat the hypotension of septic shock.

Under normal conditions, endothelium-derived nitric oxide (Figure 4) is produced by a calcium- and calmodulin-dependent nitric oxide synthase (117-119). Signal transduction pathways, linked to cell-surface receptors for vasodilators such as acetylcholine and histamine, control nitric oxide production by this constitutive isoform of nitric oxide synthase. Inflammatory mediators induce a calcium-independent form of nitric oxide synthase in endothelial cells (120) and vascular smooth muscle (121). In contrast to constitutive nitric oxide synthase, its induced form is inherently activated (121) and is not controlled by receptor-dependent mechanisms. This induced form may increase nitric oxide production in septic patients (122) and in patients given IL-2 for cancer treatment (123).

Increased production of nitric oxide during septic shock may lead to several harmful effects. Nitric oxide may be largely responsible for sepsis-induced hypotension (124, 125), and in vitro studies have implicated nitric oxide in sepsis-induced myocardial depression (126). However, nitric oxide synthase inhibition has not been shown to prevent endotoxin-induced myocardial depression in vivo (127). Nitric oxide also has well-documented cytotoxic effects (128, 129), and its overproduction in septic shock could lead to direct tissue injury and organ failure. Finally, recent in vitro evidence has shown that nitric oxide may exert a proinflammatory effect during septic shock by enhancing cytokine release from phagocytic cells (130).

Despite this potential to cause harm, nitric oxide may also have beneficial effects in septic shock. It appears to play a role in maintaining visceral and microvascular blood flow (131-134). Mediators released during sepsis, such as thromboxane and endothelin-1, cause vasoconstriction, and nitric oxide production may be an important counter-regulatory mechanism (135). Further, nitric oxide’s ability to block platelet aggregation (114) and leukocyte adhesion (115) helps prevent microvascular stasis and thrombosis (134). In support of this hypothesis, the nitric oxide synthase inhibitor N^\text{-}-nitro-L-arginine increased accumulation of platelets and neutrophils in the pulmonary circulation of rabbits given cell activators (135). Another potential concern in sepsis is the effect of nitric oxide synthase inhibition on host defense because of the antimicrobial activity (134) and immunomodulating effects (130) of nitric oxide.

Nonetheless, because hypotension during sepsis is an important predictor of organ injury and death, use of nitric oxide synthase inhibitors may improve survival in septic shock by increasing mean arterial pressure. As many as 50% of patients who die from septic shock have hypotension refractory to vasopressors (136). Nitric oxide synthase inhibitors restored the responsiveness of the septic vasculature to catecholamines in endotoxin-challenged animals (137). In addition, the ability of nitric oxide synthase inhibition to “normalize” mean arterial pressure in anesthetized animals challenged with endotoxin or TNF without causing hypertension (124, 125) suggests that compared with conventional therapy, nitric oxide synthase inhibitors might represent a less hazardous treatment approach. These considerations have led to the use of nitric oxide synthase inhibitors to treat hypotension in patients with sepsis (138, 139) and in those receiving cytokine therapy for cancer (140).

Although these agents can alter mean arterial pressure, beneficial effects on clinical outcomes, including survival, have not been shown convincingly in either animal or patient investigations. One study in endotoxin-challenged rats showed that partial nitric oxide synthase inhibition improved survival (141), and nitric oxide synthase inhibition also prevented the development of a “premorbid” state in dogs challenged with IL-2 (140).

In other animal models, inhibition of nitric oxide production is clearly harmful. Administration of N^-methy-L-arginine (L-NMA), a nitric oxide synthase inhibitor, to anesthetized rats and dogs increased renal vascular resistance (142) and decreased renal blood flow (143). Further, in models of sepsis using an endotoxin challenge, L-NMA increased capillary leak and intestinal damage in rats (144) and depressed cardiac output in...
anesthetized dogs (132). High doses (300 mg/kg) of L-NMA in anesthetized rats given endotoxin caused cardiovascular collapse and death (141). These data suggest that complete inhibition of nitric oxide synthase may be undesirable. Available inhibitors affect both constitutive and induced nitric oxide synthase and may interfere with the homeostatic functions of nitric oxide (141, 145).

Recently, we used nitric oxide synthase inhibitors to perform a series of studies in endotoxemic dogs. We administered Nω-amino-L-arginine (L-NAA) continuously for 22 hours to healthy and endotoxemic dogs at two doses (1 mg/kg per hour and 10 mg/kg per hour) (127). In this model, L-NAA increased the systemic vascular resistance index but decreased the cardiac index, the oxygen delivery index, and the oxygen consumption index. In contrast to previous reports of anesthetized animals (125), nitric oxide synthase inhibition in conscious animals had less effect on mean arterial pressure in endotoxin-challenged animals than in healthy controls. The reason for this difference is not clear, but anesthetic agents decrease mean arterial pressure and change hemodynamic parameters in complex ways that may interact with the effects of endotoxin and nitric oxide synthase inhibitors (146). Unexpectedly, at doses that did not substantially increase mean arterial pressure, L-NAA decreased survival time (127). In addition, high doses of L-NAA given to healthy dogs caused muscular rigidity and seizure-like activity. Whether the decreased survival time in endotoxemic animals was due to nitric oxide synthase inhibition or to idiosyncratic toxicities of L-NAA was unclear.

To resolve this question, a larger but similarly designed study was conducted using several doses (1, 2, 4, and 10 mg/kg per hour) of another nitric oxide synthase inhibitor, L-NMA (147), which has been given to patients with septic shock (138) and to patients with cancer who were receiving IL-2 (140). In endotoxemic animals, L-NMA increased the systemic vascular resistance index but decreased the cardiac index and oxygen delivery index, increased lactic acidosis, produced hepatic toxicity, and, at the highest dose examined (10 mg/kg per hour), increased the mortality rate. The effects of nitric oxide synthase inhibition on the cardiac index observed in this study were similar to those found in other animal models (124, 125, 127, 132, 148, 149) and in patients with sepsis (138, 139). The findings increase the concern that these agents may decrease tissue perfusion in septic shock. Some authors report that L-NMA increases endotoxin-induced liver injury in other models, and some propose that nitric oxide may protect the liver during sepsis (150).

Nitric oxide can exert harmful and beneficial effects during septic shock. Only nonselective nitric oxide synthase inhibitors that block the constitutive and induced forms of the enzyme have been studied extensively. These nonselective nitric oxide synthase inhibitors have not proved beneficial in the treatment of septic shock. In the future, nitric oxide synthase inhibitors that are highly selective for the induced isoform of the enzyme or for particular vascular beds will probably be developed and will warrant further investigation.

Discussion

Dr. Charles Natanson: As the participants in this conference have shown, none of the therapies aimed at single targets in the inflammatory cascade have proved to be safe and effective for treating septic shock in humans. Dr. Hoffman showed that antibodies directed at core epitopes of bacterial endotoxin failed to protect humans against the harmful effects of sepsis (8, 10, 30–34, 39, 40, 44). At least one antiendotoxin antibody discussed produced harm (44, 46), perhaps because of its nonspecific binding affinity (28, 46). Despite decades of research, it has never been shown clearly that targeting this single exogenous toxin is a viable strategy to treat a complex inflammatory response to diverse gram-negative bacteria. However, newer agents (see Table 1) are being developed that may have more potent antiendotoxin activity in vitro and in vivo than core-directed antibodies. Testing of these agents may finally determine whether endotoxin is a useful therapeutic target in septic shock.

Therapies directed at endogenous inflammatory mediators have also failed to produce unequivocal benefit in sepsis. Dr. Suffredini presented data from multiple clinical trials showing that inhibition of cytokines was not beneficial (73, 80–83) and that one anti-TNF therapy was harmful (81). Dr. Eichacker discussed data that suggest that, in a model of severe peritonitis-induced septic shock, inhibiting neutrophil function may have adverse effects, whereas augmenting it may be beneficial (103, 105, 110). Dr. Danner showed that during severe experimental endotoxemia, nonselective inhibition of nitric oxide production provides no substantial benefit and may be harmful (127).

A recent study in C3-deficient dogs provides further insight into the potential dangers of altering the natural balance of inflammatory mediators (151). Activation of complement by endotoxin has been implicated in the pathogenesis of septic shock. Unexpectedly, C3-deficient dogs, when challenged with an E. coli endotoxin, had decreased endotoxin clearance, worsened shock, and organ failure. The third component of complement has cleavage products (C3a and C5b) that are critical to both inflammatory reactions and host defense. These data remind us that inflammatory mediators perform important functions, such as clearing bacterial toxins, that must not be impaired if anti-inflammatory therapies are to be successful. Perhaps our therapeutic premise has been flawed: Attempting to block the harmful effects of inflammatory mediators may not produce a net benefit because it may also compromise host defense and ultimately worsen outcome.

Although animal studies performed in our laboratories have not shown the benefit of inhibiting inflammatory mediators, other laboratories showed that some of these agents were efficacious and provided a strong scientific rationale for therapies directed at specific targets in the septic cascade (7, 55, 56, 66, 74–76). However, many of these animal models use an intravenous bolus of bacteria or bacterial toxins in an otherwise healthy animal. This experimental design may not resemble the development of infection in patients, which commonly occurs over days and probably begins with a measured re-
Figure 5. Pathogenesis of septic shock divided by level of inflammatory response.

Response to a local infection. In animals, release of inflammatory mediators in response to a rapid infusion of microbial toxins may be exaggerated and produce more harm than benefit. The bolus model may be better suited to illustrate potentially pathologic events in sepsis than to show the therapeutic benefit of a particular therapy. For example, anti-TNF antibodies provided protection in intravenous bolus models but were not beneficial in an experimental peritonitis model (77, 79) and, in some studies, were actually harmful (78). In this case, the peritonitis model may produce results more readily extrapolated to infectious diseases in humans.

Animal models are, by necessity, highly controlled. Animals are of similar age and health and are treated identically, receiving the same microbial challenge. The heterogeneity of patients with sepsis may help explain the divergence between preclinical and clinical results discussed in this conference. The diversity of age (from neonates to the elderly), underlying disease (cancer to collagen-vascular disease), and types of infection (pneumonia to meningitis) found in these patients is extraordinary. It has confounded researchers and clinicians trying to design, interpret, and compare clinical trials. Further, in contrast to animal studies, many factors can influence clinical outcome in a patient with sepsis, from the quality of medical care received to the site of infection, virulence of infecting microorganism, underlying disease, and, perhaps most important, immunocompetence.

Many patients with sepsis are immunocompetent, such as otherwise healthy patients with urinary tract infections or pneumonia. They often respond well to standard antibiotics and cardiovascular support and have an adequate inflammatory response that controls the infection and eliminates microbial toxins (Figure 5). Anti-inflammatory therapies may not benefit such patients and could impair the body’s ability to fight infection. An excessive inflammatory response may develop in other patients, who have not been well characterized (58, 60). Anti-inflammatory treatments might reduce their risk for tissue injury and decrease mortality, as has been hypothesized (5-7, 55, 56, 58, 60, 66, 74-76). However, to improve outcome without compromising the essential protective functions of the host defense system, clinicians must learn exactly when treatment must be initiated and which mediators must be inhibited, determine the degree of inhibition, and identify appropriate patients. This therapeutic approach is ambitious and its feasibility remains unproved.

Finally, some patients with sepsis are immunocompromised by steroid administration, neutropenia, and genetic or acquired immune deficiencies and may have an inadequate inflammatory response to infection. For them, augmented host defense mechanisms may promote clearance of bacteria and their toxins and reduce tissue injury. In the future, successful therapeutic approaches may depend on determining which inflammatory mediators should be inhibited or augmented and when to do so. Laboratory tests that can rapidly measure levels of specific bacterial toxins or host mediators may be needed to identify and treat patients according to their particular immune responses. Use of anti-inflammatory agents in sepsis and septic shock is clearly more complex than first believed.

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