Clinical Sepsis Trials*
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Despite advances in antibiotic therapy and the care of critically ill patients, mortality rates for sepsis have remained high, with death rates of greater than 40 percent in patients presenting with septic shock. Great progress has been made recently in identifying the biochemical mediators associated with infection which can produce organ system dysfunction, hypotension, and death in this setting. In particular, macrophages activated by bacterial products, including endotoxin derived from Gram-negative bacteria, appear to occupy a central role in producing the physiologic disregulation associated with severe infection. These activated macrophages release increased levels of proinflammatory cytokines, including interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and IL-6.

Elevated serum levels of endotoxin, IL-1, TNF-α, and IL-6 are associated with increased mortality in patients with sepsis. In animal models of endotoxemia or bacteremia, blockade of the actions of TNF-α or IL-1 with monoclonal anticytokine antibodies or with antagonists which competitively bind to the cytokine receptor, such as the IL-1 receptor antagonist (IL-1ra), results in improved survival. In contrast, IL-6 appears to have a less important role in the pathogenesis of sepsis-induced organ system dysfunction, even though it does appear to be a relatively good predictor of outcome in critically ill patients with sepsis.

Large multicentered studies recently have been completed which examined the efficacy of therapies aimed at blocking the effects of endotoxin, TNF-α, and IL-1 in patients with sepsis. In each study, patients were enrolled who had clinical evidence of infection associated with organ system dysfunction or shock or both. In particular, entry criteria generally were permutations of the recently defined "sepsis syndrome" and "septic shock" classifications of patients. Although none of these studies showed therapeutic benefit among the total group of patients enrolled, subgroups of patients appeared to show improved outcome, suggesting that interventions aimed at modifying macrophage activation may have utility in some patients suffering from severe infection.

Antiendotoxin Therapies

Monoclonal antibodies to the lipid A component of endotoxin were utilized in at least four large studies. One of the antiendotoxin antibodies (E5) was produced in mice, and the other (HA-1A) was largely of human origin. In initial studies, neither antibody showed efficacy among all patients enrolled, but HA-1A appeared to reduce mortality in patients with Gram-negative bacteremia whether hypotensive or not, with decreases in mortality of approximately 40 percent in these groups. Treatment with E5 was not associated with improvement in patient survival, but did appear to decrease organ system dysfunction in patients with Gram-negative infections, whether bacteremic or not. Subsequent studies, however, were unable to duplicate these initial results.

At least one reason for the lack of efficacy of the antilipid A antibodies in clinical settings appears to be their relatively weak ability to block the activating effects of endotoxin on macrophage release of proinflammatory cytokines such as TNF-α. In in vitro studies, even if high concentrations of E5 or HA-1A are added to supernatants containing endotoxin, these supernatants are still able to activate macrophages to produce TNF-α, demonstrating that these antibodies have only weak antiendotoxin blocking ability.

Other antiendotoxin agents, such as bactericidal/permeability increasing protein (BPI), have potent actions in blocking the effects of endotoxin in vitro and in vivo. In particular, infusion of BPI in animals treated with endotoxin results in markedly improved survival. Initial clinical studies with BPI are underway. It has a short serum half-life, and this may be a factor limiting its clinical utility.

Anti-TNF Therapies

In animal models of endotoxemia, Gram-negative, or Gram-positive bacteremia, therapy with agents able to block the effects of TNF, such as monoclonal anti-TNF antibodies or soluble TNF receptors, results in markedly improved survival. In a small phase I/II study, monoclonal anti-TNF antibodies produced in mice were found to be safe, and treatment with this antibody appeared to be associated with improved survival in patients with elevated circulating TNF levels. There also appeared to be improvement in cardiac function, particularly in left ventricular stroke work index, in septic patients treated with anti-TNF antibodies.

A large phase II/III clinical study in patients with sepsis syndrome with or without shock has recently been completed. This study found no benefit from therapy with anti-TNF antibodies in patients with clinical evidence of infection and organ system dysfunction, but without shock. In contrast, there was a 17 percent reduction in mortality for patients with septic shock. Because two doses of the antibody (7.5 mg/kg and 15 mg/kg) were examined vs placebo in this anti-TNF trial, a statistically significant benefit from therapy was not able to be proven in the patients with septic shock. A second study is currently in progress to better define the role of anti-TNF therapy in patients with septic shock.

Another approach to blocking the effects of TNF has centered on using soluble TNF receptors able to bind and inactivate TNF prior to the interaction of TNF with its cellular receptor. By combining the extracellular portions of two TNF receptors with an immunoglobulin...
Fc component, a TNF-binding construct (rhu TNFR:Fc) was created with long serum half-life and the ability to bind TNF approximately 30 times as well as the monoclonal anti-TNF antibody. Because this soluble TNF receptor construct is totally of human origin, no antibody response to the construct appears to develop after injection.

In animal models of endotoxemia, administration of rhu TNFR:Fc up to 2 h after endotoxin still produces improved survival. A phase II clinical study of sTNFR:Fc in patients with clinical evidence of infection and shock has recently been completed, but full analysis of the data from this trial is not yet available.

Interleukin-1 Receptor Antagonist

The interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring interleukin-1 antagonist which binds to both the type I and type 2 IL-1 receptors without cellular activation. IL-1ra competitively inhibits the actions of IL-1 and appears to play an important role in counterbalancing the proinflammatory properties of IL-1 in disease states such as arthritis and chronic infections. In animal models of endotoxemia and Gram-negative and Gram-positive infection, infusion of IL-1ra is associated with improvement in hemodynamics and survival.

A large international multicentered placebo-controlled trial examined two doses of IL-1ra (1 mg/kg/h and 2 mg/kg/h) infused continuously over 72 h in patients presenting with clinical evidence of infection with organ system dysfunction and/or shock. In this study, no benefit was found for IL-1ra among all patients enrolled or in patients presenting in shock. However, in a retrospective analysis using a mortality predictive index based on APACHE III variables, an approximately 22 percent reduction in mortality was found in patients with a predicted mortality rate greater than 24 percent who were treated with IL-1ra at either dose level. A study aimed at confirming this apparent improvement in survival associated with IL-1ra therapy in patients with high predicted mortality is presently underway. The current IL-1ra study is of particular interest because it is the first sepsis study to use a predictive index of mortality with a defined "cut-off point" in deciding which patients will be entered into the trial to receive therapy. A potential concern with the design of the current IL-1ra trial is the implication that if the drug is shown to be effective, then it will be marketed with a mortality predictor which must be calculated before patients will be able to receive this therapeutic agent.

Other Clinical Trials

Other interventions aimed at mediators believed to be important in contributing to organ system dysfunction and mortality in septic patients are being actively investigated in clinical trials. In a double-blinded placebo controlled trial of BN 52021 (a platelet activating factor antagonist) involving 262 patients, no improvement in mortality was seen in the overall patient group. However, in a subgroup of patients with documented Gram-negative infection, this agent appeared to reduce mortality by approximately 42 percent (from 57 percent to 33 percent in the treated group).

In experimental models of endotoxemia and Gram-negative bacteremia, cyclo-oxygenase inhibition improved hemodynamics and outcome. A multicentered trial investigating intravenous ibuprofen, primarily in patients with ARDS, is nearing completion. Similarly, increased production of bradykinin may contribute to hemodynamic compromise in sepsis. In endotoxemic rats, therapy with a bradykinin antagonist improved survival, and this agent, CP 027, is now being investigated in a large, multicentered, phase II/III clinical study.

Conclusions

Despite the impressive experimental findings of improved survival when bacteremic or endotoxemic animals are treated with anticytokine therapies, endotoxin-binding compounds, or other agents directed against important mediators of organ system dysfunction in sepsis, clinical trial results to the present have shown no clearcut benefit for the use of these agents, with reductions in mortality suggested only in patient subgroups, which were defined retrospectively. The only exception to this retrospective identification of subgroups was the anti-TNF monoclonal antibody study where patients with septic shock were identified prospectively. Unfortunately, as mentioned above, although a trend towards benefit was seen with anti-TNF therapy in patients with septic shock, this did not achieve statistical significance. Clearly, animal models for sepsis, which generally use acute endotoxin or bacterial insults in a previously healthy animal, differ markedly from the clinical situation, where infection usually has no well-defined starting time and occurs in patients with pre-existent medical problems. In addition to these differences in models, the results from the clinical sepsis trials force a rethinking of the paradigm that a single mediator can play a central role contributing to organ system dysfunction and mortality in sepsis. Rather, the relatively modest improvement in outcome, even in subgroups of patients, achieved with monoclonal anti-TNF antibodies or IL-1ra demonstrates the necessity of defining biochemical and clinical markers albeit to better identify patients who may benefit from these therapies. Such an approach was suggested in the initial phase I/II monoclonal anti-TNF antibody study, where patients with elevated serum TNF levels appeared to have a better response to the antibody, as defined by survival from their septic episode. Additionally, results from the recently completed clinical studies, showing evidence for increased circulating levels of additional proinflammatory cytokines, despite the blockade of IL-1 or TNF-α, indicate that combination therapy may be useful in achieving further decreases in mortality among patients with sepsis, organ system dysfunction, and shock.

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**Endotoxin, Endotoxin-Binding Protein, and Soluble CD14 Are Present in Bronchoalveolar Lavage Fluid of Patients With Adult Respiratory Distress Syndrome**


A circulating lipopolysaccharide-binding protein (LBP) binds endotoxin and activates macrophages via the CD14 receptor on the macrophage surface. We have shown that this system is relevant in the lungs, as LBP significantly reduces the concentration of LPS required to activate human alveolar macrophages, and a specific monoclonal antibody to CD14 blocks the effect of LBP/LPS complexes on alveolar macrophages. To investigate the potential importance of the LBP/LPS/CD14 system in the lungs, we measured LPS, LBP, and soluble CD14

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