Therapies for Sepsis

Emerging Therapies for Sepsis and Septic Shock

Discussant
EDWARD ABRAHAM, MD, *Denver, Colorado*

These proceedings have been transcribed from selected Grand Rounds of the Department of Medicine at Stanford University Medical Center by the presenter(s) or author(s) and by Edward D. Harris Jr, MD, George DeForest Barnett Professor of Medicine and Associate Editor, THE WESTERN JOURNAL OF MEDICINE. *The data and opinions presented in these edited transcripts are those of the presenter(s) and do not necessarily reflect the views of the faculty at Stanford University Medical Center.*

EDWARD D. HARRIS JR, MD: *This Medical Grand Rounds is being presented by Edward Abraham, MD. After receiving both his BA and MD degrees from Stanford University, Dr Abraham obtained his medical residency and critical care–emergency medicine fellowship training in the University of California, Los Angeles (UCLA), system. After serving on the faculty at UCLA, he joined the faculty at the University of Colorado School of Medicine in 1993. He has received support from the National Institutes of Health for his studies of hemorrhage-induced suppression of the immune response and he is the principal investigator of a critical care treatment group for the study of the treatment of the adult respiratory distress syndrome. Since 1994, funding for his clinical trials has included support from Sentron Medical, Synergen Corporation, The Liposome Company, Miles Pharmaceuticals, Cell Therapeutics, Hoffmann-LaRoche Incorporated, Zeneca Limited, Alliance Pharmaceutical Corporation, and the Bayer Corporation. We have asked Dr Abraham to update us on the emerging therapies for sepsis and septic shock.*

EDWARD ABRAHAM, MD*: The challenges of performing and interpreting clinical trials are perhaps illustrated best by recent efforts to test antagonism to mediators of septic shock. One of the major lessons learned from these studies is the importance of doing thorough basic research before taking on clinical trials. Without this firm bedrock, much money can be spent, patients exploited, and years wasted. Data from recently completed clinical trials, however, provide reasons for optimism about where we are finally heading in the field of cytokine antagonism. We appear to be on the verge of making meaningful and major advances in improving survival from overwhelming sepsis and septic shock.

*Professor of Medicine, University of Colorado School of Medicine; Section Head, Critical Care Medicine; and Director, Medical Intensive Care Unit, University Hospital, Denver.

About 400,000 patients each year have severe sepsis and septic shock in the United States. Of these, it is estimated that 30% to 40% with severe sepsis die despite therapeutic efforts in intensive care units.[1] This rate has remained remarkably constant over the past 50 years despite improvements in therapy. The number of patients seen with septic shock and severe sepsis in intensive care units increases each year and has doubled over the past ten years. Factors in the patient population that contribute to a higher frequency of severe sepsis include older age, unscheduled surgical procedures, and comorbid medical diseases.

In recent years there has been increased interest in the role of activated lymphocytes and macrophages in generating the pathologic features accompanying sepsis. In particular, attention has been focused on the cytokines that are abundantly expressed in sepsis. These inflammatory mediators include tumor necrosis factor (TNF), interleukin (IL)-1, IL-8, and IL-6. Levels of these mediators increase markedly in patients with sepsis. Several bacterial products such as endotoxins from gram-negative organisms, exotoxins, and other products from gram-positive bacteria, viruses, and fungi may activate macrophages and initiate the cytokine cascade.

When *Escherichia coli* is infused into baboons, the increase in proinflammatory mediators occurs quickly.[2] The levels of TNFα, IL-6, IL-8, and IL-1β all rise in the peripheral circulation and even higher at local sites in organs such as the lungs, liver, and intestines.

What happens when a person is given endotoxin? Can endotoxin cause hemodynamic abnormalities? Can it reproduce what we see in septic patients? In a series of experiments performed at the National Institutes of Health on volunteers who had Swan-Ganz catheters and arterial lines placed, endotoxin infusions were administered.[3] Blood pressures dropped by more than 25%, the cardiac index increased by 60%, and there was a 50% decrease in peripheral vascular resistance despite a 2-

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Reprint requests to Edward Abraham, MD, Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado Health Sciences Center, 4200 E Ninth Ave, Denver, CO 80262.

ABBREVIATIONS USED IN TEXT

BPI = bactericidal permeability–increasing [protein]
Ig = immunoglobulin
IL = interleukin
IL-1ra = IL-1–receptor antagonist
TNF = tumor necrosis factor

liter intravenous infusion of normal saline solution halfway through the experiment. In summary, when normal healthy persons are given endotoxin, the result is what is seen in intensive care units: shock, high cardiac output, and low peripheral resistance.

What about endogenous mediators, those produced in severe inflammatory states, including sepsis? In rabbits given TNF, hemodynamic alterations are produced that are similar to those found after endotoxin infusion: decreases in blood pressure and peripheral vascular resistance, with increases in cardiac output and heart rate.[4] If TNFα and IL-1β are infused simultaneously, there is a synergistic response. Amounts of each of these cytokines that are insufficient alone to cause an effect produce substantial hemodynamic changes when given together.

Additional pieces of data linking IL-1 and TNF to the hemodynamic changes seen in patients with sepsis come from a number of studies that show a direct relationship between plasma levels of these cytokines and the likelihood of death. For example, TNF levels in patients who died of sepsis were shown to be substantially higher from day 1 on than in those who survived.[5] The IL-1 data are similar, but there is less difference between survivors and nonsurvivors.[6]

Therapeutic Approaches to Septic Shock

Many modalities of therapy have been aimed at trying to improve survival of patients with severe sepsis and septic shock. The mainstays of therapy include fluid, nutrition, antibiotics, and ventilatory support. Blood pressure support is added using catecholamines. Recent studies suggest that better blood pressure support can be achieved by using nitric oxide synthase inhibitors,[7] but the possible and actual toxicity of these is unclear at present.

Before I discuss recent studies of novel therapeutic agents for sepsis, it may be useful to revisit the fate of a previously popular therapy for sepsis that in double-blind studies was shown to be of no benefit and even harmful in some cases: giving glucocorticoids to patients with severe sepsis and septic shock. Two studies examined this treatment.[8,9] The results of one of them is shown in Figure 1. There was no difference in the overall mortality in patients who received large doses of methylprednisolone sodium succinate compared with those receiving placebo at the time of entry. But in a subgroup of patients who had elevated creatinine levels and who were administered glucocorticoids, there was an increased frequency of shock, a decreased percentage of shock reversal, and a statistically significant increase in death rates. At this time, the use of high doses of glucocorticoids as therapy for septic shock cannot be recommended.

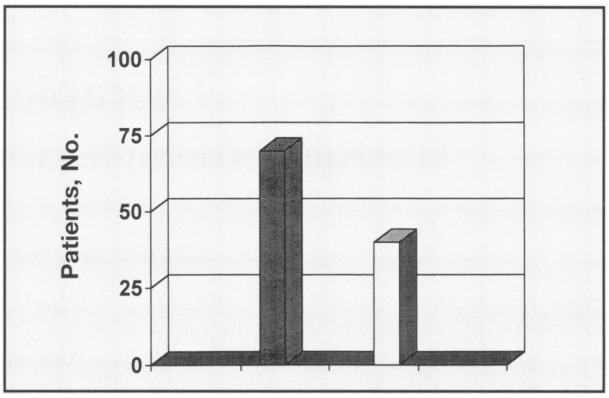

Figure 1.—Mortality is shown in patients with sepsis with creatinine levels of greater than 180 μmol per liter (>2.0 mg per dl) and treated with either high-dose glucocorticoids (stippled bar) or placebo (white bar) (adapted from the Veterans Administration Systemic Sepsis Cooperative Study Group[9]).

In the past several years, a series of studies have examined the possible blockade of mediators in the proinflammatory cascade that accompanies severe infection. The agents examined include monoclonal antibodies to endotoxin, expected to help in patients who have infection with gram-negative organisms, antibodies, and soluble receptor complexes that block TNFα, which should be helpful against most bacteria, fungal and viral organisms, and IL-1 inhibitors, such as the IL-1–receptor antagonist (IL-1ra). This receptor antagonist would also be expected to be beneficial in patients with bacterial, viral, or fungal infections.

Interest in blocking the effects of endotoxin increased when the results of a study were published using antiserum produced in human subjects to the J5 endotoxin from *E coli*.[10] Subsequently, monoclonal immunoglobulin (Ig) M antibodies to the lipid A component of endotoxin were generated and examined in clinical trials of septic patients. The initial human study of antilipid A antibodies showed no efficacy when the entire cohort who had infusions of the antiendotoxin antibodies was examined.[11] A retrospective subgroup analysis, however, showed a 39% reduction in mortality in patients with severe gram-negative bacteremia and an even larger difference in patients in whom shock due to gram-negative infections developed.

Before antiendotoxin antibodies were licensed for general use, a second study was done using these antibodies (known as HA-1A).[12] No benefit could be shown, and in fact, a trend toward increased mortality in patients infected with non–gram-negative organisms was noted in patients who had received the antibody.

A variable not adequately considered before the human studies were done is that these antiendotoxin antibodies do not block endotoxin with high affinity. In a series of cellular in vitro experiments that were published after the clinical studies were completed, the effects of these antibodies on macrophages in cell cul-

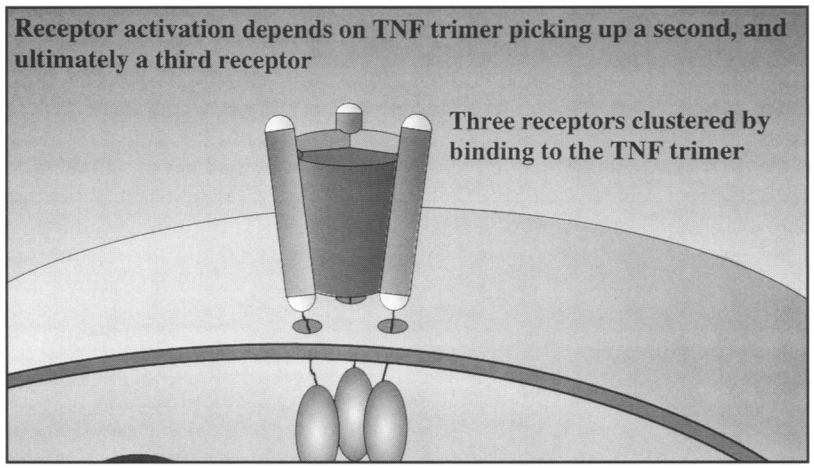

Figure 2.—A schematic of an activated tumor necrosis factor (TNF) receptor complex is shown: TNFα circulates as a trimer and initiates intracellular signaling events through clustering of TNF receptors on the cell surface.

ture were examined both before and after endotoxin was put into the system.[12] In the cultures that received antibodies against endotoxin, there was no decrease in the release of IL-1, TNFα, or IL-6 after endotoxin was added to the macrophages. This was in contrast to the effective blockade induced by using a true antiendotoxin agent, polymyxin B.

The in vitro experiments with HA-1A are in contrast to studies using bactericidal permeability–increasing (BPI) protein, an endogenous endotoxin-binding peptide. In animals given BPI at various times after the exposure to endotoxin, there is an improvement of survival to endotoxin challenge. If neutropenic rats are given *Pseudomonas* species organisms and then treated with BPI, there is an improvement in survival.[13] The BPI protein is being used in phase I clinical trials and appears to be safe, although large doses must be given by continuous infusion, and the agent may be expensive.

An important potential problem with using antiendotoxin agents in clinical situations is getting drug to the patients early enough. By the time the active compound would be given, endotoxemia has already induced the production of IL-1 and TNFα and has initiated a proinflammatory response. The benefit of blocking endotoxin at this clinical stage, when increased proinflammatory cytokine expression has already occurred, remains unknown.

The IL-1–receptor antagonist binds to the IL-1 receptor, has no agonist actions of its own, and blocks the effects of IL-1.[14] In experiments on animals, survival from bacteremia is improved after therapy with IL-1ra.[15] In these experiments, rabbits were infused with *E coli* and then treated with IL-1ra. The fall in blood pressure in untreated animals is blunted, but not ablated, by IL-1ra. This decrease in blood pressure is probably related to the release of TNFα whose effects would not be affected by anti–IL-1 therapy.

The IL-1–receptor antagonist has been examined in three clinical trials. The first was an unblinded, placebo-controlled study of 99 patients and showed a greater than 50% reduction in mortality in patients who received this agent.[16] The second was a double-blind, placebo-controlled study involving 893 patients.[17] Two doses of IL-1ra were tested against placebo. Although no benefit was seen in the overall group of patients enrolled in the clinical trial, a retrospective data analysis of subgroups based on 28-day all-cause mortality suggested that patients with a predicted mortality of greater than 24% derived some benefit from IL-1ra. Unfortunately, this is a difficult analysis to perform at the bedside. Physicians would need to distinguish between patients with a predicted mortality of 22%, who should not get the drug, and those with a predicted mortality of 26%, who should receive it.

A third study with IL-1ra was then done.[18] It was hypothesized that based on organ system dysfunction, decisions could be made about predicted mortality and which patients should or should not receive the drug. That study was entirely negative and was stopped at the interim analysis. There was no evidence of benefit in the overall patient group or in the more severely ill patients. Of note, after the study was completed, the effects of IL-1ra in a group of human subjects who had been given lipopolysaccharide infusions with and without IL-1ra were reported.[19] In those experiments, few indices of inflammation were altered in the IL-1ra–treated subjects. In particular, the release of TNFα and other cytokines was not affected by IL-1ra therapy in persons with endotoxemia. Had these experiments been done before the clinical trials, it might have been predicted that IL-1ra would have been of minimal efficacy in endotoxin-induced inflammation. Subsequently, IL-1ra has been abandoned as an alternative therapy in patients with severe sepsis and septic shock.

Anti–Tumor Necrosis Factor-α Therapy

Tumor necrosis factor-α circulates as a molecular trimer, and because of this it is able to generate clustering of its receptors on cell surfaces, leading, after bind-

ing, to intracellular signal transduction. When TNFα binds two receptors, one level of signal transduction is induced; when three receptors are activated, the reaction is markedly enhanced. The implications of these data are that any therapy against TNF must bind at least two of the three components of the TNFα trimer to be effective. If two parts of the trimer are left exposed, then receptor coupling, receptor clustering, and activation can still occur (Figure 2).

The initial anti-TNF therapies examined were monoclonal antibodies against TNFα. Experiments in baboons had shown a benefit of therapy with these monoclonal antibodies in animals that received infusions of *E coli.*[20] In untreated animals, shock intervened quickly and the animals died. At autopsy, diffuse neutrophilic infiltration was found in multiple organs. In the animals who received monoclonal antibodies against TNFα, there was only a minimal dip in blood pressure, associated with survival from the septic insult. Of particular interest is the fact that treatment with anti-TNFα after either *E coli* or gram-positive organisms are infused still results in an improvement in survival.[21]

In a phase II-III study,[22] a murine monoclonal anti-TNFα IgG1 antibody with a half-life of about 50 hours was used. Two different doses, 7.5 and 15 mg per kg of body weight, were tested against placebo in this three-arm study of patients with shock and nonshock sepsis. Eligibility criteria for inclusion in the study consisted of hyperthermia, tachycardia, tachypnea, and at least one of the following indicators of organ dysfunction: decreased level of consciousness, hypoxemia consistent with the acute respiratory distress syndrome, metabolic or lactic acidosis, decreased urine output, or disseminated intravascular coagulation. Patients did not have to be in shock to be eligible for inclusion in this study, but there was a prospective randomization of patients into shock and nonshock groups. In addition, there was a time line: Patients had to be identified within 12 hours of the development of organ system dysfunction, and they then had another 4 hours to get drug. All patients were observed for the next 28 days.

The principal outcome variable in the anti-TNF monoclonal antibody study was mortality from all causes at day 28, so that presumably a patient who was well enough to leave the hospital on day 10 and then died in an automobile accident on day 20 would be included as a death in the study. A total of 994 patients were enrolled, and no serious side effects were noted. In all patients included in the study, there was no significant difference in mortality. When we look at the prospectively defined groups, however, a totally different picture emerges. There was no beneficial effect associated with anti-TNF therapy in patients without hypotension. In contrast, in shock patients, there is a substantial difference in outcome between the placebo-treated group and the patients administered the monoclonal antibody (Figure 3). In patients receiving anti-TNFα compared with placebo, mortality in the treated group was decreased 50% at day 3, 30% at day 14, and 17% at day 28.

A second study (INTERSEPT) was carried out in Europe that compared the use of this monoclonal antibody in two doses (3 and 15 mg per kg) against placebo.[23] In the INTERSEPT clinical trial, 420 patients in shock were enrolled. Outcomes were similar to those of the North American study: about a 14.5% decrease at day 28 in the patients treated with the lower dose of monoclonal antibody.

The other strategy for blocking the actions of TNFα uses soluble TNF receptors as competitors for binding circulating TNFα. There are two TNF receptors on the cell surface: one a lower molecular weight p55 receptor (also called type 1) and the other a higher molecular weight p75 (type 2) receptor. Both of these transduce signals intracellularly after binding with TNF. Both receptors bind TNF with high affinity.

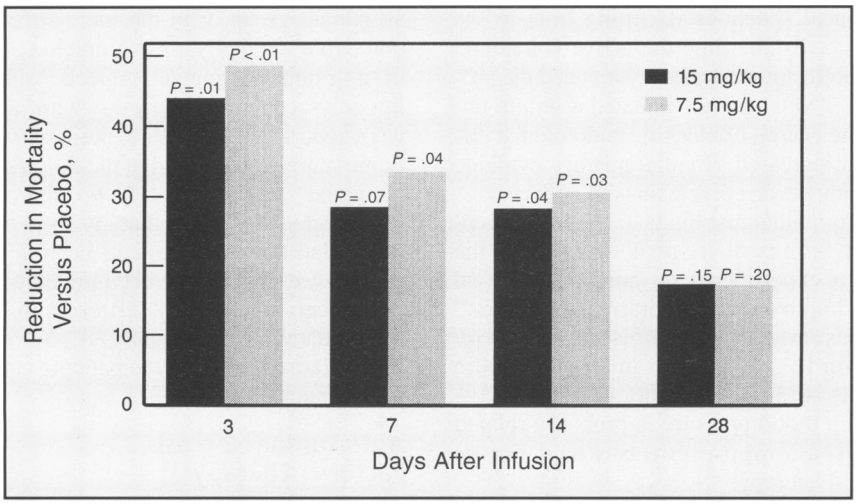

Figure 3.—The relative reduction in mortality is shown at the indicated postinfusion time points in patients in septic shock treated with monoclonal anti–tumor necrosis factor-α antibodies compared with placebo (from Abraham et al[22]).

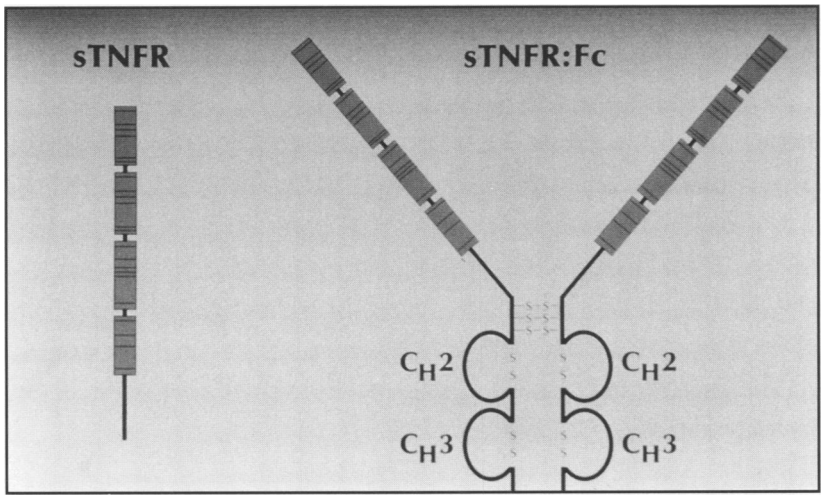

Figure 4.—The structure of the tumor necrosis factor receptor **(TNFR)**– immunoglobulin G1:Fc fusion protein is shown.

The sequence for the extracellular component of the human TNF receptors has been joined to the Fc portion (Figure 4) of a human IgG1 molecule and transfected into human cell cultures. The resulting construct is a 100% human protein. These soluble TNF-receptor:Fc constructs are able to bind two of the three components of TNF and thereby block TNF binding to its receptors. In addition, they bind TNF at more than 100 times the affinity of monoclonal antibodies, implying that a 100-fold lower dose of the soluble receptor construct could be used to block TNFα.

Two studies have examined the efficacy and safety of therapy with TNF-receptor fusion proteins in patients with sepsis. The first clinical trial investigated a receptor construct using the type 2 receptor.[24] When the study was designed, it was known from work with the monoclonal TNF antibody that patients with septic shock appeared to respond to anti-TNF therapies. Therefore, in the study using the p75 TNF-receptor fusion protein, patients enrolled were in shock, did not necessarily have organ system dysfunction, and had treatment initiated within 24 hours of going into shock. Results showed that mortality rose from 32% in the placebo-treated group to 53% ($P = .01$) in the patients who received the highest dose of this soluble TNF receptor construct.

In a subsequent study, the type 1 p55 TNF-receptor:Fc construct was used at much lower doses, so that it was calculated that 95% of the bioavailable TNF would be blocked for a period of less than three days after infusion.[25] This study compared three doses of the p55 TNF-receptor fusion protein with placebo and incorporated two prospectively defined groups of patients. At the conclusion of the study it was found that in the overall group of patients, there was a minimal, statistically insignificant decrease in day 28 mortality with the highest dose of the p55 TNF-receptor fusion protein. In the first of the two prospectively defined subgroups, that is, patients with refractory shock requiring pressor therapy for more than two hours and organ system dysfunction, no substantial benefit (or harm) was seen with p55 TNF-receptor fusion protein therapy. In contrast, in patients with dysfunction of at least two organ systems (severe sepsis), with or without early septic shock, there was a 36% reduction in mortality at day 28 ($P = .07$).

Why were such remarkable differences seen in outcome in patients treated with the p55 and the p75 TNF-receptor fusion proteins? There are at least three possible reasons for the markedly different results with these two compounds: First, the dose of the p75 TNF-receptor fusion protein that was associated with the greatest increase in mortality, 1.5 mg per kg, was almost 20-fold greater than the highest dose of the p55 TNF-receptor fusion protein used, 0.08 mg per kg. It is certainly possible that blocking all the effects of TNF, which is an important mediator of the inflammatory response, for prolonged periods may result in harm because an intact inflammatory response is a necessary component of normal host defense. Second, it is possible that the p75 TNF-receptor fusion protein was used in patients who were not sufficiently ill to benefit from such therapy and, in fact, were harmed by the interruption of normal inflammatory pathways. In the placebo group of the p75 receptor fusion protein study, the mortality was about 30%, and it is possible that immunosuppression in this group of insufficiently ill patients had deleterious effects. Finally, there apparently are important biologic differences between the p55 and the p75 TNF-receptor fusion protein complexes, with the p75 TNF-receptor fusion protein acting as a "carrier" for TNF, whereas the p55 TNF-receptor fusion protein more completely blocks the actions of TNFα.[26]

We can conclude from the completed clinical trials that blocking TNF in a group of patients who are critically ill with organ system dysfunction, but without refractory septic shock, can improve mortality. This hypothesis is being tested in several ongoing clinical tri-

als, and the results from these studies should be available soon.

We are at an exciting time in developing new therapies for critically ill patients. Although biotechnologically derived products did not appear to realize their initial promise, continued investigation with these molecules has identified a group of therapies that have the potential for substantially improving the survival of patients with overwhelming infection. Should the anti-TNF therapies indeed show benefit in critically ill patients, a new era in the management of such patients will begin where improvement in survival and the resolution of organ system dysfunctions, including such problems as the acute respiratory distress syndrome, can be achieved through the modulation of immune responses.

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