

TREATMENT OF SEPTIC SHOCK WITH THE TUMOR NECROSIS FACTOR RECEPTOR:Fc FUSION PROTEIN

CHARLES J. FISHER, JR., M.D., JAN M. AGOSTI, M.D., STEVEN M. OPAL, M.D., STEPHEN F. LOWRY, M.D., ROBERT A. BALK, M.D., JERALD C. SADOFF, M.D., EDWARD ABRAHAM, M.D., ROLAND M.H. SCHEIN, M.D., ERNEST BENJAMIN, M.D., FOR THE SOLUBLE TNF RECEPTOR SEPSIS STUDY GROUP*

Abstract Background. A recombinant, soluble fusion protein that is a dimer of an extracellular portion of the human tumor necrosis factor (TNF) receptor and the Fc portion of IgG1 (TNFR:Fc) binds and neutralizes TNF- α and prevents death in animal models of bacteremia and endotoxemia.

Methods. To evaluate the safety and efficacy of TNFR:Fc in the treatment of septic shock, we conducted a randomized, double-blind, placebo-controlled, multicenter trial. A total of 141 patients were randomly assigned to receive either placebo or a single intravenous infusion of one of three doses of TNFR:Fc (0.15, 0.45, or 1.5 mg per kilogram of body weight). The primary end point was mortality from all causes at 28 days.

Results. There were 10 deaths among the 33 patients

in the placebo group (30 percent mortality), 9 deaths among the 30 patients receiving the low dose of TNFR:Fc (30 percent mortality), 14 deaths among the 29 receiving the middle dose (48 percent mortality), and 26 deaths among the 49 receiving the high dose (53 percent mortality) ($P=0.02$ for the dose-response relation). Baseline differences in the severity of illness did not account for the increased mortality in the groups receiving the higher doses of TNFR:Fc.

Conclusions. In patients with septic shock, treatment with the TNFR:Fc fusion protein does not reduce mortality, and higher doses appear to be associated with increased mortality. (*N Engl J Med* 1996;334:1697-702.)

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SEVERE sepsis causes substantial morbidity and mortality among critically ill patients. Despite advances in critical care, the incidence of sepsis continues to increase, with a mortality rate of approximately 40 percent.^{1,2} The 13th most common cause of death in the United States, septic shock results in an estimated 100,000 deaths per year.

Experimental and clinical data have shown that the proinflammatory cytokines tumor necrosis factor α (TNF- α) and interleukin-1 are important mediators of severe sepsis. TNF- α is present in the systemic circulation after the administration of live or heat-killed bacteria or endotoxin.^{3,4} Administration of TNF- α reproduces many of the physiologic and laboratory changes associated with severe sepsis,⁵⁻⁹ and antibodies against TNF- α have a protective effect in animal models of severe sepsis.¹⁰⁻¹⁵

The effects of TNF- α are mediated through 60-kd (type I) and 80-kd (type II) cell-surface TNF receptors. The extracellular portions of the receptors are shed in vivo and then bind circulating TNF- α , thereby blocking its bioavailability.¹⁶⁻¹⁹ The plasma concentration of cir-

culating free TNF receptor is correlated with disease activity in inflammatory conditions.^{20,21} The presence of TNF- α and its ratio to soluble TNF receptor in plasma are correlated with mortality from sepsis.²²⁻³¹

In the present study, we evaluated the efficacy of a dimeric form of the type II TNF receptor linked with the Fc portion of human IgG1 (TNFR:Fc) in patients with septic shock. This fusion protein provides protection against death in animal models of gram-positive and gram-negative bacterial sepsis.³²⁻³⁵ In normal subjects, intravenous administration of TNFR:Fc in doses ranging from 1 to 60 mg per square meter of body-surface area was safe and attenuated the cytokine and leukocyte responses to endotoxin.³⁶

METHODS

The trial was conducted in 15 academic medical centers in the United States. The primary end point was mortality from all causes at day 28 after administration of the study drug. Secondary end points included resolution of organ failure, time to death, time to discharge from the intensive care unit, and time to discharge from the hospital. Patients were stratified for the severity of illness at base line according to the overall score on the Acute Physiology and Chronic Health Evaluation (APACHE) II, the acute-physiology score on APACHE III,³⁷ and plasma cytokine (TNF- α , interleukin-1 β , and interleukin-6) and endotoxin concentrations. The protocol was approved by the institutional review board at each participating center, and written informed consent was obtained from all patients.

Study Design and Treatment

Patients were randomly assigned in a double-blind fashion to receive placebo or one of three doses of TNFR:Fc (0.15, 0.45, or 1.5 mg per kilogram of body weight) administered as a single 100-ml infusion over a period of 30 minutes. Recombinant human TNFR:Fc is composed of two molecules of the extracellular portion of the 80-kd TNF receptor covalently linked to the hinge region of human immunoglobulin IgG1. The fusion protein retains the Fc portion of IgG1 but lacks the CH1 region of the immunoglobulin. The dimeric TNFR:Fc fusion protein is then expressed in Chinese-hamster-ovary cells. TNFR:Fc has an affinity for TNF- α at a level of 10^{-10} M. The placebo, identical in appearance to the fusion protein, consisted of the buffer without

From the Department of Pulmonary and Critical Care Medicine, Cleveland Clinic Foundation, Cleveland (C.J.F.); the Research and Development Division, Immunex Corporation, Seattle (J.M.A.); the Department of Medicine, Brown University School of Medicine and Memorial Hospital of Rhode Island, Pawtucket (S.M.O.); the Department of Surgery, Cornell University Medical Center, New York (S.F.L.); the Sections of Pulmonary and Critical Care Medicine, Rush-Presbyterian-St. Luke's Medical Center, Chicago (R.A.B.); the Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, D.C. (J.C.S.); the Department of Medicine, University of California, Los Angeles (E.A.); the Department of Medicine, University of Miami and Veterans Affairs Medical Center, Miami (R.M.H.S.); and the Department of Surgery, Mt. Sinai Medical Center, New York (E.B.). Address reprint requests to Dr. Fisher at the Critical Care Research Unit, Department of Pulmonary and Critical Care Medicine, Mail Code G-62, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195.

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*The members of the Soluble TNF Receptor Sepsis Study Group are listed in the Appendix.

TNFR:Fc. Decisions about antimicrobial drug therapy, supportive care, and surgical intervention were made by the patients' attending physicians and were not dictated by the study protocol.

Selection of Patients

The definition of septic shock was based on standard clinical definitions.³⁸ The criteria for enrollment included the following findings within the previous 24 hours: fever or hypothermia (temperature, $\geq 38.2^{\circ}\text{C}$ or $\leq 36.0^{\circ}\text{C}$), tachycardia (heart rate, ≥ 90 per minute), tachypnea (respiratory rate, ≥ 20 per minute; arterial partial pressure of carbon dioxide, ≤ 32 mm Hg; or the need for mechanical ventilation), and hypotension despite adequate fluid resuscitation (systolic blood pressure, ≤ 90 mm Hg; mean arterial pressure, ≤ 65 mm Hg; a sustained decrease in systolic pressure of ≥ 40 mm Hg; or the need for vasopressors [except < 5.0 μg of dopamine per kilogram per minute]).

Patients who were less than 18 years old, pregnant, or organ-transplant recipients and those with hemorrhagic or cardiogenic shock were not enrolled. Other exclusion criteria were infection with the human immunodeficiency virus, treatment with corticosteroids (the equivalent of ≥ 1 mg of prednisone per kilogram) within the previous 48 hours, neutropenia, participation in an ongoing investigational clinical trial, and the presence of irreversible underlying disease anticipated to be rapidly fatal.

Evaluation of Patients

All patients were followed throughout the 28-day study period or until death occurred. Samples of blood and other suspected sites of infection were obtained for culture within 72 hours before or after the administration of the study medication.

The primary source of infection, causative pathogen, and adequacy of antimicrobial therapy were determined in a blinded fashion by a critical care specialist and an infectious-disease specialist, according to prospectively defined criteria.^{39,40} Adequate antimicrobial therapy was defined as the administration of at least one drug to which the causative organism was susceptible within 24 hours after the onset of sepsis. *Pseudomonas pneumonia* required at least two active drugs and polymicrobial infections in the abdomen required an antimicrobial drug active against enteric anaerobic bacteria.

APACHE II scores and APACHE III acute-physiology scores were measured at the time of enrollment. Plasma obtained before the infusion of the study drug and 24, 48, and 72 hours afterward was tested for TNF- α , interleukin-1 β , and interleukin-6 by enzyme-linked immunosorbent assays (ELISA) (ImmuneX, Seattle) and was tested for endotoxin by the quantitative turbidimetric assay (Associates of Cape Cod, Woods Hole, Mass.). The TNF assay detects TNF bound to receptor (inactive form) and unbound TNF (active form). Serum samples for measurements of anti-TNFR:Fc antibodies (by indirect-antibody ELISA) were obtained before the infusion of the study drug and on day 28. A cytokine-endotoxin score was calculated from base-line values for TNF- α , interleukin-1 β , interleukin-6, and endotoxin, according to previously described methods.⁴¹

Statistical Analysis

All data were analyzed according to a prospectively defined analytic plan, unless otherwise specified. The analyses included data from all patients who were randomized (intention-to-treat analysis). Demographic and base-line characteristics were analyzed with the Cochran-Mantel-Haenszel test to assess the comparability of the groups with respect to factors possibly related to the outcome.

The primary end point was mortality from all causes on day 28. The data were analyzed for a dose-response relation between TNFR:Fc and mortality and for an effect in all three groups of treated patients, as compared with those receiving placebo. These analyses were performed with the Cochran-Mantel-Haenszel test (1 df). In the dose-response analysis, the increments between doses were considered equivalent, and a test for linear trend was performed.

Secondary end points were compared with use of the log-rank test. In the analysis of time to discharge from the intensive care unit or hospital, data on patients who died during the study period were censored (i.e., the patients were considered to be hospitalized and in the

Table 1. Base-Line Characteristics of 141 Patients with Septic Shock Assigned to Receive Placebo or One of Three Doses of TNFR:Fc.*

CHARACTERISTIC	STUDY GROUP			
	PLACEBO (N = 33)	0.15 mg/kg (N = 30)	0.45 mg/kg (N = 29)	1.5 mg/kg (N = 49)
Age (yr)	60 \pm 3	52 \pm 3	62 \pm 3	62 \pm 2
Weight (kg)	73.8 \pm 4.1	81.3 \pm 4.7	80.0 \pm 3.7	77.3 \pm 2.9
	<i>percentage of patients</i>			
Male sex	42	60	69	63
White race	67	60	59	71
Underlying disease				
Coronary artery disease	21	27	35	31
Alcoholism	24	23	28	27
Chronic renal failure	15	17	28	20
Chronic liver failure	18	20	14	20
Diabetes	18	20	24	14
Nonhematologic cancer	15	23	21	16
Immunosuppressive disorder	15	23	10	6
Chronic obstructive pulmonary disease	0	17	0	22
Recent surgery	42	37	34	45

*Base-line characteristics did not differ significantly among the groups, with the exception of chronic obstructive pulmonary disease. Plus-minus values are means \pm SE. Some patients had more than one underlying disease.

intensive care unit) at 28 days. The APACHE II scores, APACHE III acute-physiology scores, and plasma cytokine values were analyzed descriptively.

Multiple logistic-regression analysis was performed to evaluate the effect of imbalances in base-line characteristics among the groups and to determine the extent to which any imbalances were associated with the observed treatment effect. The following base-line variables, defined at the outset of the study, were evaluated by multiple logistic-regression analysis: sex, weight, vasopressor requirements, gram-negative cause of sepsis, gram-positive cause of sepsis, bacteremia, urinary tract infection, log plasma endotoxin value, log plasma endotoxin values squared, log plasma interleukin-6, interleukin-1 β , and TNF- α values, acute respiratory distress syndrome, disseminated intravascular coagulation, hepatobiliary insufficiency, acute renal failure, persistent shock, any organ failure, APACHE II score, and APACHE III acute-physiology score.

RESULTS

Comparison of the Study Groups

All 141 patients received the study medication in accordance with the protocol, except for one patient in whom the administration of the medication was interrupted because of transient hypotension. Table 1 shows the distribution of base-line characteristics among the four groups. The underlying diseases did not differ significantly among the groups, with the exception of chronic obstructive pulmonary disease, which was present in 17 patients who received 0.15 mg of TNFR:Fc per kilogram and in 22 who received 1.5 mg per kilogram but in none of the patients in the other two groups ($P=0.002$). The anatomical sources of sepsis did not differ significantly among the groups, although there were fewer skin or wound infections in the TNFR:Fc groups than in the placebo group. The causative organisms also did not differ significantly among the groups, although there were more patients with *pseudomonas*

Table 2. Causative Organisms of Severe Sepsis in Patients Treated with TNFR:Fc.*

ISOLATE	STUDY GROUP			
	PLACEBO (N = 33)	0.15 mg/kg (N = 30)	0.45 mg/kg (N = 29)	1.5 mg/kg (N = 49)
	<i>percentage of patients</i>			
<i>Escherichia coli</i>	6	13	24	10
<i>Klebsiella</i>	6	3	10	8
<i>Enterobacter</i>	3	3	3	6
<i>Pseudomonas</i>	3	0	14	4
<i>Proteus</i>	0	3	0	8
Other gram-negative organisms	9	10	17	10
Anaerobes	3	3	0	0
<i>Staphylococcus aureus</i>	0	3	7	10
Coagulase-negative staphylococcus	9	13	17	10
<i>Streptococcus pneumoniae</i>	3	10	7	4
<i>Enterococcus</i>	9	7	14	14
Other gram-positive organisms	9	13	17	16
Yeast	12	23	7	14
Unknown	24	13	13	20

*Differences among the study groups were not significant. Some patients had more than one causative organism.

infection in the group receiving 0.45 mg of TNFR:Fc per kilogram than in the other three groups ($P=0.08$), and there were more patients with gram-positive causes of sepsis in the three TNFR:Fc groups than in the placebo group (Table 2).

The severity of sepsis at enrollment did not differ significantly among the four groups (Table 3). Antibiotic therapy was judged to be adequate in 91 percent of the placebo recipients and in 92 percent of the TNFR:Fc recipients.

Efficacy of TNFR:Fc

There was a dose-response relation between treatment with TNFR:Fc and mortality ($P=0.02$) (Fig. 1). Comparison of the placebo group with all three TNFR:Fc groups combined showed no significant difference in mortality ($P=0.13$).

Bacteremia was less frequent in the placebo group (in 27 percent of the patients) than in the low-dose group (37 percent), middle-dose group (48 percent), and high-dose group (43 percent), although the difference was not statistically significant ($P=0.35$, with 3 df). Among the 55 patients who had bacteremia during the course of the study, it was detected in 78 percent within 24 hours after enrollment. When the analysis was controlled for the presence of clinically important bacteremia at enrollment, the dose-response relation between TNFR:Fc and mortality was still significant ($P=0.03$).

There was a trend toward increased rates of mortality with higher doses of TNFR:Fc among the patients with gram-positive organisms (13, 29, 50, and 62 percent in the placebo, low-dose, middle-dose, and high-dose groups, respectively). In patients with gram-negative organisms alone or polymicrobial infections, treatment

with TNFR:Fc did not have an adverse effect. The difference in the trend in mortality rates between the patients with gram-positive infections and those with gram-negative or polymicrobial infections was statistically significant ($P=0.02$), but there were too few patients to determine whether this result was of clinical relevance.

Median plasma interleukin-6 and endotoxin concentrations at base line are shown in Table 4. There was a statistically significant dose-response relation between TNFR:Fc and mortality after the analysis had been adjusted for the base-line plasma interleukin-6 and endotoxin concentrations and the cytokine-endotoxin score.⁴¹

Logistic-regression analysis identified the following base-line predictors of mortality at 28 days: dose of TNFR:Fc ($P<0.001$), APACHE II score ($P<0.001$), log base-line plasma interleukin-6 concentration ($P<0.002$), and presence of acute respiratory distress syndrome ($P<0.01$) or disseminated intravascular coagulation ($P<0.01$). The adverse association of TNFR:Fc with mortality at 28 days could not be explained by base-line differences in these variables. There were no differences among the groups in the onset of organ failure or bacterial infections three or more days after treatment, time to death, or time to discharge from the intensive care unit or hospital.

There were no clinically important antibody reactions to TNFR:Fc. Thirteen of 84 follow-up samples (15 percent) were positive by ELISA for antibodies to TNFR:Fc; however, 4 of the positive samples were from the 22 patients in the placebo group (18 percent), suggesting a significant false positive rate. TNF- α was detected in plasma at base line in only 5 of the 141 patients (4 percent). Free TNF- α is rapidly cleared from plasma. Yet in the presence of TNFR:Fc, TNF- α binds to the TNF receptors, and the receptor:ligand complex is retained in the plasma. This form of TNF is measurable by ELISA even though it is no longer biologically

Table 3. Severity and Complications of Sepsis at Enrollment in Patients Treated with TNFR:Fc.*

VARIABLE	STUDY GROUP			
	PLACEBO (N = 33)	0.15 mg/kg (N = 30)	0.45 mg/kg (N = 29)	1.5 mg/kg (N = 49)
APACHE II score	25.2 \pm 1.3	23.9 \pm 1.5	24.5 \pm 1.3	25.8 \pm 1.0
APACHE III acute-physiology score	94.9 \pm 5.0	88.2 \pm 5.8	91.2 \pm 5.4	99.8 \pm 4.0
	<i>percentage of patients</i>			
Complications				
Acute respiratory distress syndrome	15	20	28	22
Acute renal failure	33	27	41	45
Hepatobiliary insufficiency	24	23	31	33
Disseminated intravascular coagulation	24	23	24	31
Persistent shock	54	67	69	65

*Differences among the study groups were not significant. Plus-minus values are means \pm SE. Some patients had more than one complication of sepsis. APACHE denotes Acute Physiology and Chronic Health Evaluation.

active. After the administration of TNFR:Fc, TNF- α was detected in plasma by ELISA in 40 percent of the patients in the three dose groups.

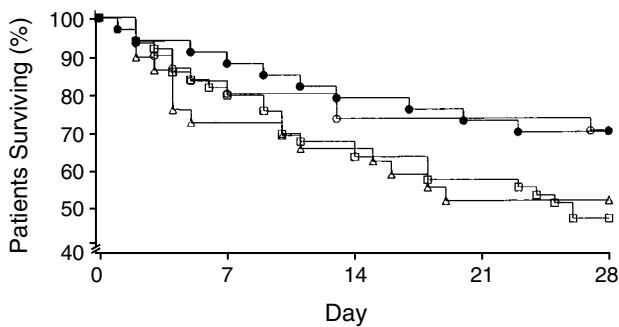
The plasma interleukin-6 concentration, a marker of the activation of inflammatory cytokines, was analyzed for evidence of late cytokine release. Very few patients had elevated interleukin-6 values late in the study, and there was no correlation between these values and the dose of TNFR:Fc.

DISCUSSION

The increased mortality rate among patients treated with TNFR:Fc as compared with placebo was unexpected. There are several possible explanations for this result. Randomization may have resulted in imbalances in prognostic factors among the four groups at enrollment. A detailed multivariate analysis, however, failed to demonstrate an imbalance of sufficient magnitude to account for the unfavorable results.

Three other explanations may account for the unfavorable results. TNFR:Fc may have had a toxic effect; the complete removal of TNF- α may have been deleterious; or the fusion protein may have functioned as an intravascular carrier of TNF- α , prolonging the inflammatory response.

It has been postulated that the isotype of the fusion peptide may cause some form of Fc-mediated immu-



STUDY GROUP	NO. OF PATIENTS	NO. OF DEATHS
Placebo (●)	33	10
0.15 mg/kg (○)	30	9
0.45 mg/kg (△)	29	14
1.5 mg/kg (□)	49	26

Figure 1. Kaplan-Meier Analysis of Survival in Patients with Sepsis Receiving Placebo or One of Three Doses of TNFR:Fc. An intention-to-treat analysis of mortality from all causes at 28 days by the Cochran-Mantel-Haenszel test showed a dose-response relation between treatment with TNFR:Fc and mortality ($P=0.02$). Mortality did not differ significantly between the placebo group and the three treatment groups combined ($P=0.13$).

Table 4. Base-Line Plasma Cytokine and Endotoxin Concentrations.*

VARIABLE	STUDY GROUP			
	PLACEBO	0.15 mg/kg	0.45 mg/kg	1.5 mg/kg
Detectable tumor necrosis factor α (% of patients)†	0	10	0	4
Detectable interleukin-1 β (% of patients)‡	6	10	11	19
Interleukin-6				
Detectable (% of patients)§	65	63	64	69
Median (pg/ml)	310	393	455	304
Range (pg/ml)	<156–35,620	<156–140,000	<156–330,000	<156–160,000
Endotoxin				
Detectable (% of patients)¶	61	67	83	65
Median (pg/ml)	120	150	140	110
Range (pg/ml)	7.9–1530	9.0–4290	9.1–1540	8.9–1110
Cytokine-endotoxin score (% of patients)				
0–2	52	37	28	49
4–6	46	53	59	41
8–10	0	10	14	10

*Differences among the study groups were not significant. A total of 137 patients were tested for cytokines, and 131 for endotoxin.

†Range, 84 to 269 pg per milliliter (lower limit of detection, 78).

‡Range, 111 to 651 pg per milliliter (lower limit of detection, 78).

§Lower limit of detection, 156 pg per milliliter.

¶Lower limit of detection, 5 pg per milliliter.

||For an explanation of the scoring system, see Casey et al.⁴¹

notoxicity, which may explain the adverse effects of TNFR:Fc.⁴² There is no direct evidence from our study to support this possibility. TNFR:Fc does not activate complement in *in vitro* assays (unpublished data).

Clinical trials have demonstrated the safety of TNFR:Fc and its marked efficacy in ameliorating the clinical symptoms of rheumatoid arthritis.⁴³ There was no evidence of disease exacerbation or immunotoxicity in patients with arthritis receiving TNFR:Fc. Similarly, we observed no direct toxicity of TNFR:Fc in our patients with sepsis. Their clinical course was characteristic of that of severe sepsis, and the excess mortality was accounted for by the progression of the septic process.

Many animal models show complete ablation of TNF- α by TNFR:Fc, yet they show an overall benefit with respect to outcome.³²⁻³⁴ In one study, treated animals had prolonged elevation of plasma TNF- α at values much lower than the initial peak values.⁴⁴ Since peak concentrations of TNF- α in patients with sepsis, if detectable at all, generally range from 100 to 1000 pg per milliliter, the late release of TNF- α at minimally elevated levels would presumably have a negligible clinical effect. The TNF- α ELISA used in our study detects both free and receptor-bound TNF- α and so could not be used to distinguish between the two forms. Plasma interleukin-6 concentrations, which are a useful indicator of activation of the inflammatory cascade, did not increase after TNFR:Fc treatment. There were no clinical observations that suggested that TNF- α was released late, although late release would be difficult to detect clinically. There was no difference between the placebo and treatment groups in the onset of organ failure after the administration of the study medication.

In normal subjects challenged with endotoxin and

treated with TNFR:Fc at doses similar to those used in this trial, TNFR:Fc was safe and TNF- α disappeared from the systemic circulation.³⁶ There was no late release of TNF- α , and serum samples from the subjects neutralized exogenous TNF- α at concentrations up to 10,000 pg per milliliter.³⁶

TNF- α is essential to the generation of both innate and acquired immune responses to an infectious challenge. In animal models of salmonella or systemic listeria infection, inhibition of TNF- α or interleukin-1 may worsen the outcome.⁴⁵⁻⁴⁷ Treatment with anti-TNF- α antibody has been shown to be either ineffective or detrimental in mice with localized peritonitis.^{48,49} TNF- α or interleukin-1 may actually protect endotoxin-hyporesponsive C3H/HeJ mice during systemic *Escherichia coli* infection.⁵⁰ In our study, TNFR:Fc may have effectively removed circulating TNF- α , resulting in an exacerbation of the systemic infection in some patients. The dimeric nature of the TNFR:Fc molecule results in greater avidity for binding^{32,51,52} than that of previously tested monoclonal antibodies.^{13,53,54}

In a previous trial of anti-TNF- α monoclonal antibody for the treatment of sepsis, there was a nonsignificant trend toward higher mortality at 28 days in the subgroups of patients without shock (20 percent in the placebo group, 22 percent in the group receiving 7.5 mg per kilogram, and 24 percent in the group receiving 15 mg per kilogram).⁵³ In a second trial of the same antibody, the high dose (15 mg per kilogram) resulted in a trend toward increased overall mortality (40 percent in the placebo group and 42 percent in the high-dose group).⁵⁴ The mortality in the subgroups of patients without shock was 26 percent in the placebo group and 37 percent in the high-dose group. If the elimination of TNF- α activity exacerbates infection in patients with sepsis, the safety of TNF- α inhibition in the management of sepsis will require serious reconsideration.

In this trial there was no adverse effect of treatment in patients with gram-negative sepsis. However, there was a strong trend toward increased mortality with higher doses in patients with gram-positive infections. Two recent studies of platelet-activating factor antagonists and the first phase 3 study of interleukin-1-receptor antagonists showed that patients with gram-positive infections were not benefited and were possibly harmed by treatment with these antimediator agents.⁵⁵⁻⁵⁷

APPENDIX

The following centers and investigators participated in the Soluble TNF Receptor Sepsis Study: *Baylor College of Medicine* — J.L. Zimmerman and P. Allee; *Beth Israel Hospital, Boston* — M.P. Fink and F. Favorito; *Cleveland Clinic Foundation* — T.H. Seifert; *Cornell University Medical Center* — S.M. Coyle; *Massachusetts General Hospital* — B.T. Thompson and F. Favorito; *Memorial Hospital of Rhode Island* — P.K. Dubin; *Mt. Sinai Medical Center* — T.J. Iberti, E. Benjamin, R. Del Giudice, and J. Jones; *Rush-Presbyterian-St. Luke's Medical Center* — C. Hill and L. Butler; *University of California, Davis, Medical Center* — G.E. Foulke and W.F. Walby; *University of Florida, Jacksonville* — B.W. Meyers, P. Fuqua, and E. Ventresca; *University of California, Los Angeles, Medical Center* — C. Perry and P. Bellamy; *University of Michigan Medical Center* — R. Fekety and J. Kugler; *University of Washington Medical Center* —

R.K. Root, M. Stark, and P. Dellenger; *Veterans Affairs Medical Center, Miami* — M. Pena and M. Wasserlauf; and *Veterans Affairs Medical Center, Boise* — D.L. Stevens and S. Gaffigan. *Immune Research and Development* — R. Hanna, A. Hendricks, S.L. Krause, M. Lange, W.H. Lounsbury III, A. Rubin, and S. Scheeler. *Consulting statistician* — J. Wittes, Statistics Collaborative, Washington, D.C.

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