Effects of recombinant dimeric TNF receptor on human inflammatory responses following intravenous endotoxin administration.

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Effects of dimeric TNF receptor (p80) Fc (TNFR:Fc) on acute phase responses were evaluated in 18 volunteers given endotoxin (4 ng/kg i.v.). Subjects were randomized to receive either placebo (n = 6), low dose TNFR:Fc (10 mg/m² i.v., n = 6), or high dose TNFR:Fc (60 mg/m² i.v., n = 6). TNFR:Fc blocked plasma TNF bioactivity (p = 0.001) and increased, in a dose-ordered fashion, TNF immunoreactivity (p < 0.001). TNFR:Fc decreased secondary cytokine levels including IL-1β (p = 0.007), IL-8 (p < 0.001), IL-1 receptor antagonist (p < 0.001), granulocyte-CSF (p = 0.03), and growth regulated peptide-α (p = 0.001) but not macrophage inflammatory protein-1α or IL-10. Low dose, but not high dose, TNFR:Fc blunted or delayed the release of epinephrine and cortisol (p ≤ 0.026). Despite the absence of plasma TNF bioactivity, high dose TNFR:Fc was less immunosuppressive than low dose TNFR:Fc as measured by cytokine and stress hormone responses. Endotoxin-related symptoms were not altered by TNFR:Fc and the febrile response was delayed but not diminished (p = 0.004). Increases in cardiac index (72 ± 19%) and heart rate (60 ± 10%) and decreases in systemic vascular resistance index (47 ± 7%) were unaltered by TNFR:Fc. These data suggest that the inflammatory response to endotoxin can escape from high levels of circulating TNF by anti-TNF Abs or dimeric TNF receptor fusion proteins (2). The latter approach to improve outcome in septic shock is to modulate this inflammatory response by neutralizing circulating TNF with anti-TNF Abs or TNF receptor fusion proteins (2). The latter class of antagonists was developed by fusing the gene for the extracellular portion of the TNF receptor (either the type I, 55-60 kDa, or type II, 75-80 kDa receptor) with the Fc domains of IgG (6-10). The fusion protein binds TNF with a high affinity and cell signaling is prevented by inhibition of the cross-linking of cell-associated receptors (6, 9-11). One such product consisting of two identical TNF p80 receptors linked by the Fc fragment of the IgG1 molecule (TNFR:Fc) binds TNF with a 50-fold greater affinity (Kᵢ = 10¹⁰ M⁻¹) than monomeric receptor (10).

Inhibition of TNF by anti-TNF Abs or dimeric TNF receptors has been protective in some animal models of sepsis or endotoxemia (7, 9, 10, 12-14). These beneficial effects, however, are not universal and TNF antagonists are detrimental in models of peritonitis or infections due to intracellular pathogens, suggesting a critical role for TNF in host defenses (15-19). Human trials of TNF antagonists in sepsis have shown no overall survival benefit (2, 20-23) and in one trial, TNFR:Fc unexpectedly increased mortality (21). The lack of efficacy of TNF inhibitors has not been fully explained but these findings question the concept that therapy directed against TNF will limit the harmful inflammatory responses that occur during human septic shock. The current hypothesis of modulating inflammatory responses during sepsis may require reevaluation.

The administration of i.v. endotoxin to humans is a model of the cardiovascular and inflammatory responses that occur during sepsis (24, 25). TNFR:Fc was evaluated in this model to study the effects of neutralizing circulating TNF on the inflammatory responses and clinical manifestations of endotoxemia. The data show that this intervention was not entirely anti-inflammatory, was associated with unexpected interactions, and it did not interrupt the clinical consequences of endotoxin challenge. The observed alterations in the inflammatory response provide a potential explanation for some of the deleterious effects of this experimental therapy in patients with sepsis (21).

**Methods**

**Subjects and study plan**

Eighteen healthy nonsmoking volunteers (14 men and 4 women, 19 to 37 yr, 25 ± 1 yr, mean ± SEM) participated in the study. All underwent nont human TNF; HuTNFR-M1, human type II TNF receptor; IL-1ra, IL-1 receptor antagonist.

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3 Abbreviations used in this paper: TNFR:Fc, TNF p80 receptors linked by the Fc fragment of the IgG1 molecule; MIP-1α, macrophage inflammatory protein; GR-α, growth-regulated peptide; G-CSF, granulocyte-CSF; rTNF, recombinant human TNF; HuTNFR-M1, human type II TNF receptor; IL-1ra, IL-1 receptor antagonist.
complete histories and physical examinations and had normal electrocardiograms, chest radiographs, and blood and urine analyses. The study was approved by our institutional review board on human experimentation and written, 0.5-μg LPS obtained from each subject. Study participants were admitted to the medical intensive care unit after fasting overnight and given maintenance i.v. fluids.

The subjects were randomized to receive either TNFR:Fc vehicle (placebo, n = 6), low dose TNFR:Fc (10 mg/m², n = 6), or high dose TNFR:Fc (60 mg/m², n = 6). Earlier studies using these doses of TNFR:Fc in normal volunteers showed TNF bioactivities of greater than 60% and did not result in any significant clinical or laboratory abnormalities including serum Abs to TNFR:Fc (26).

The TNFR:Fc was reconstituted with 1 ml of sterile water for injection (USP) from a lyophilized powder containing 10 mg of TNFR:Fc. 1.2 mg Tris (methaneammonium salt) USP, 10 mg mannitol USP, and 40 mg mannannoside USP. The final doses of TNFR:Fc (10 mg/m² or 60 mg/m²) or placebo were diluted in 100 ml of 0.9% saline and infused for 30 minutes. Following the infusion of TNFR:Fc or placebo, purified LPS prepared from Escherichia coli 0113 (U.S. Standard Reference Endotoxin, Lot EC-5, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD) was administered (4 mg/kg of body weight, n = 19) for 1 min and flushed with 10 ml of normal saline.

Oral temperature (Diatek, San Diego, CA), respiratory rate, heart rate, and blood pressure were measured at 15-min intervals. Blood pressure was continuously monitored from radial artery catheters (Arrow International, Redding, PA). The number and severity of symptoms (chills, headache, anorexia, myalgia, and nausea) were assessed at the end of each study day. Arthritis measurements were obtained on subsequent study days. Plasma was anticoagulated with a negative control protein. To assess the neutralizing capacity of the administered mAb reactive against human type I TNF receptor (HuTNFR-M1, Immunex) and bioactivity was measured as described above (10). Normal rat serum (both Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each test well. TNF specific killing was defined as the difference in cell killing with and without TNF Ab and was compared with standard curves produced with recombinant human TNF (rR&D Systems, Minneapolis, MN) in normal human plasma. The limit of detection of the assay was approximately 1 pg/ml.

To estimate the amount of TNF bound by the administered TNFR:Fc, plasma samples obtained at 1.5 h (the usual time of peak TNF activity after endotoxin) (29) were incubated at 37°C for 30 min with 30 μg/ml of rat-neutralizing mAb reactive against human type II TNF receptor (HuTNFR-M1, Immunex) and bioactivity was measured as described above (10). Normal rat serum (equivalent to 1.5 mg/ml of IgG; Cappel, Malvern, PA) was used as a negative control. To assess the neutralizing capacity of the administered mAb reactive against human type II TNF receptor (HuTNFR-M1, Immunex) and bioactivity was measured as described above (10).

Levels of cytokines were measured in duplicate by quantitative immunoassays. Standard curves were generated using normal human plasma or sera, and samples were measured directly or diluted in plasma, serum, or urine. The limits of detection of the ELISA were as follows: TNF (R&D Systems), 15.7 pg/ml plasma; IL-10 (Biosource), 4.5 pg/ml plasma; soluble TNF receptor type I p55 (R&D Systems), 7.8 pg/ml plasma. In separate experiments, TNFR:Fc did not interfere with the detection of any of the above cytokines or receptors.

Measurements of hepatocyte (C-reactive protein and serum amyloid A), endothelial cell (soluble E-selectin), and neutrophil (lactoferrin) activation were performed (30-32). C-reactive protein was measured by radial immunodiffusion assay (The Binding Site, Birmingham, UK) with a limit of detection of 0.033 mg/dl. The limits of detection by ELISA were as follows: soluble E-selectin (British Biotechnology, Abington, UK), 0.6 pg/ml plasma; serum amyloid A (Biosource), 9.4 ng/ml serum; and lactoferrin, 1 ng/ml plasma. Lactoferrin was assayed by ELISA as previously described (33) with modifications using BSA diluted/blocking solution and ABTS peroxidase substrate (both Kazeegraad and Perry Laboratories, Gaithersburg, MD) and 96-well plates (Nunc Immunoplate Maxisorp, Naperville, IL).

Plasma ACTH hormone and cortisol levels were measured by radioimmunoassay and plasma epinephrine was measured by HPLC with electrochemical detection (SmithKline Beecham Clinical Laboratories, Owings Mills, MD).

Statistics

Data were analyzed using a three-way ANOVA model that included the following factors: 1) dose of TNFR:Fc (placebo, low, and high), 2) subject nested with dose of TNFR:Fc, and 3) time (33). In addition, a dose-time interaction was included in the model to test for different time courses in the three doses. The dose-time interaction was decomposed into two independent components: one tested the similarity of low and high dose TNFR:Fc across time and the other tested the average of low and high dose TNFR:Fc against placebo. Interactions involving subject-time were pooled into a residual term and used as the source of variability to test the time and dose-time interactions. When tests involved multiple comparisons, the p values were corrected using a Tukey procedure (33). Ordered dose effects were tested using a Bartholomew test (34). Residuals from the ANOVA model were tested using a Shapiro-Wilk test (35). In one instance (serum amyloid A) a datum was judged an outlier by the test of Dixon (36) and removed from the specific analysis.

To assess an overall difference in cytokine responses among the three groups, the mean scores of the Wilcoxon rank sum were determined for the maximum values of each cytokine and a binomial exact probability test was performed to assess the consistency of an ordering effect. Spearman correlations of minimum mean arterial pressure with maximum values of immunoactive TNF were performed. Summary data are reported as mean ± SEM.

Results

TNF and acute cytokine responses

TNF bioactivity in plasma of subjects given endotoxin was significantly blocked by TNFR:Fc administration (p < 0.001, Fig. 1A). Maximum TNF bioactivity was 154 ± 44 pg/ml in the placebo group and 8 ± 4 pg/ml in both the low and high dose TNFR:Fc subjects. In contrast, large amounts of TNF immunoreactivity were present in low and high dose TNFR:Fc groups compared with placebo (p < 0.001) and an ordered dose response was present (p < 0.001, Fig. 1B). This immunoassay measures both free and receptor-associated TNF. The latter is biologically inactive (18). At 3 h, peak TNF immunoreactivity was 7.26 ± 1.71 ng/ml and 13.02 ± 3.79 ng/ml in low and high dose TNFR:Fc subjects, respectively, and remained elevated at 24 h. The values of the placebo subjects were maximal at 1.5 h (0.466 ng/ml) and returned to detectable levels by 6 h.

To estimate the amount of TNF bound by TNFR:Fc at 1.5 h, plasma was incubated with the M1 Ab to dissociate TNF from the p80 receptor binding sites. TNF bioactivity increased in all five subjects tested (three low dose and two high dose TNFR:Fc, from
0 ng/ml to a median of 1.32 (range, 1.21 to 107.59) ng/ml. In contrast, the change in TNF bioactivity was considerably less in placebo subjects tested (n = 3), from 0.265 (0.021 to 0.543) ng/ml to 0.481 (0.326 to 0.682) ng/ml.

TNFR:Fc in 24-h plasma samples was shown to be in excess by its ability to neutralize additional TNF. Following incubation with 1 ng/ml of rhTNF, TNF bioactivity was 1.2 ± 0.47 ng/ml (placebo group, n = 3) compared with 0.010 ± 0.010 ng/ml (TNFR:Fc group, n = 3). The addition of 10 ng/ml of rhTNF to samples from four additional subjects given TNFR:Fc (two low dose and two high dose) resulted in no TNF bioactivity. Similar results were obtained using the 6-h samples from these subjects given TNFR:Fc (data not shown).

Second cytokine responses were significantly altered by the administration of TNFR:Fc (Fig. 2). Low levels of IL-1β were present in all three groups at 0 h (0 to 4 pg/ml) and only a small increase of 4.13 ± 0.65 pg/ml occurred in the placebo group while none was found in the TNFR:Fc groups (p = 0.007, Fig. 2A). In the placebo group, IL-1ra rose to maximal values at 3 h (181 ± 36.5 ng/ml) and IL-8 rose to maximal values at 2 h (2200 ± 445 pg/ml). Both of these responses were significantly decreased by TNFR:Fc administration (p = 0.001 and p = 0.001, respectively; Fig. 2, B and C). The increase in GRO-α (placebo 3 h maximum value, 305 ± 84 pg/ml) was significantly blunted by TNFR:Fc (p < 0.001, Fig. 2D). G-CSF rose in the placebo group and peak values occurred at 3 h (1586 ± 419 pg/ml). This response was significantly diminished by TNFR:Fc (p = 0.03, Fig. 2E).

Only low dose TNFR:Fc diminished IL-6 levels (p = 0.006, Fig. 2F). Peak levels of IL-6 at 2 h in the placebo group (926 ± 196 pg/ml) and high dose TNFR:Fc group (1101 ± 376 pg/ml) were similar. Levels of MIP-1α and IL-10 were not altered by TNFR:Fc administration (Fig. 2, G and H). Overall, an abnormal dose response occurred with high dose TNFR:Fc and a consistent ordering effect was found, characterized by greater suppression of maximum IL-1β, IL-1ra, IL-8, GRO-α, G-CSF, IL-6, MIP-1α, and IL-10 levels by low dose TNFR:Fc compared with high dose TNFR:Fc (p = 0.008).

Clinical, systemic, and hemodynamic responses

Endotoxin-related symptoms were unchanged by TNFR:Fc administration. All three groups experienced a similar number of symptoms (placebo 3.5 ± 0.2, low dose 4.5 ± 0.5, and high dose 2.7 ± 0.7, p = NS) and severity of symptoms (placebo 6 ± 0.6, low dose 6.1 ± 0.5, and high dose 4.3 ± 0.7, p = NS). The febrile response to endotoxin was delayed significantly by TNFR:Fc but was of similar magnitude. The maximal rise in temperature occurred at 3 h in the placebo subjects (1.47 ± 0.25°C above baseline) and at 5 h in the TNFR:Fc subjects (1.55 ± 0.18°C above baseline) (p = 0.004, Fig. 3A). Maximum heart rate (placebo 103 ± 3, low dose TNFR:Fc 100 ± 5, high dose TNFR:Fc 103 ± 3 beats/min, p = NS) and minimum mean arterial pressure (placebo 66 ± 2.4, low dose TNFR:Fc 66 ± 5.3, high dose TNFR:Fc 69 ± 3.9 mmHg, p = NS) were similar among the groups. The correlation of maximum immunoactive TNF with minimum mean arterial pressure was altered by TNFR:Fc (mean arterial pressure (placebo r = 0.77, low dose TNFR:Fc r = -0.65, high dose TNFR:Fc r = -0.48, p < 0.01) and the ordering of these correlations were similar to the abnormal dose response observed with cytokine levels.

Right-sided cardiac catheterization revealed a hyperdynamic cardiovascular response in both the placebo and high dose TNFR:Fc subjects, manifested by significant rises in heart rate and cardiac index, and significant decreases in mean arterial pressure and systemic vascular resistance index. These responses were not altered by administration of high dose TNFR:Fc. No differences were found between placebo and high dose TNFR:Fc subjects in measures of cardiac index, left ventricular ejection fraction and stroke work index, or oxygen delivery, oxygen consumption, or extraction ratio (Table I).

Acute phase responses

Leukocyte trafficking was altered significantly by TNFR:Fc (p < 0.026, Fig. 3B). The leukopenic response at 1 h in the placebo group (2.42 ± 0.3 × 10^9 cells/ml) was absent in the TNFR:Fc subjects (p = 0.005). While a subsequent leukocytosis occurred in all three groups and was of similar magnitude at 8 h, the percentage of neutrophil band forms was less (p = 0.043) (max change from baseline: placebo: 22 ± 6%, TNFR:Fc 11 ± 3%) and mature neutrophils greater (p = 0.028) (max change from baseline: placebo: 21 ± 2%, TNFR:Fc 39 ± 2%) in the TNFR:Fc subjects. The magnitude of the monocytopenia (placebo 0.9 ± 0.1%, TNFR:Fc 0.2 ± 0.2%) and lymphopenia (placebo 4.3 ± 0.5%, TNFR:Fc 3.5 ± 1%) was similar among the three groups.

The pattern of endothelial cell and neutrophil activation was also altered by TNFR:Fc. Levels of soluble E-selectin and lactoferrin were significantly diminished by TNFR:Fc (Fig. 4A, p < 0.001, and Fig. 4B, p = 0.0001, respectively). The early rise of circulating TNF receptor type I (p55) was delayed in the TNFR:Fc subjects (p = 0.0001) but by 3 h the values were similar among all three groups (Fig. 4C). At 24 h, the rise in serum amyloid A levels (baseline <25 μg/ml in both groups) was greater in the TNFR:Fc subjects (746 ± 109 μg/ml) than the placebo subjects (491 ± 61 μg/ml, p = 0.03). The rise in C-reactive protein (baseline values <20 mg/dl) at 24 h was similar among the groups (placebo 3.95 ± 0.53 vs TNFR:Fc 4.54 ± 2.7 mg/dl, p = NS).
FIGURE 2. Secondary cytokine responses (IL-1β (A), IL-1ra (B), IL-8 (C), GRO-α (D), G-CSF (E), IL-6 (F), MIP-1α (G), IL-10 (H)) following endotoxin administration in subjects randomized to receive placebo, low dose, or high dose TNFR:Fc. Data presented as mean and SEM and analyzed by three-way ANOVA.
TNFR:Fc were statistically similar and the results are pooled for presentation (see Methods). (D-7 = subjects randomized to receive placebo, low dose, or high dose TNFR:Fc. TNFR:Fc in a manner similar to the abnormal dose response that counts

**FIGURE 3.** Changes in mean temperature (A) and total leukocyte counts (B) (mean ± SEM) following endotoxin administration in subjects randomized to receive placebo, low dose, or high dose TNFR:Fc. Data analyzed by three-way ANOVA. Low dose and high dose TNFR:Fc were statistically similar and the results are pooled for presentation (see Methods). (D-7 = days before endotoxin; D7 and D14 = 7 and 14 days after endotoxin given, respectively).

Stress hormone responses were altered by the administration of TNFR:Fc in a manner similar to the abnormal dose response that was seen with secondary cytokine levels. Stress hormones were suppressed less by high dose compared with low dose TNFR:Fc (Fig. 5). Plasma epinephrine rose by 2 h in both the placebo and high dose TNFR:Fc subjects and returned to baseline by 4 to 6 h. In contrast, subjects given low dose TNFR:Fc had a significantly blunted response (p = 0.026). Cortisol levels rose in both the placebo and the high dose TNFR:Fc subjects by 2 h and this response was significantly delayed in the low dose TNFR:Fc subjects (p = 0.02). In the placebo group, ACTH rose significantly from baseline (0 h: 21.3 ± 5.8 pg/ml) to maximum values at 2 h (222 ± 40 pg/ml) and statistically similar responses occurred in both TNFR:Fc groups (p = NS).

**Discussion**

Major alterations occurred in the sequence and magnitude of the normal acute phase response to endotoxin following neutralization of circulating TNF by TNFR:Fc. Secondary cytokine levels were diminished, leukocyte margination and neutrophil recruitment was inhibited, and early activation of endothelial cells and neutrophils was blunted. However, fever, symptoms, cardiovascular responses, cortisol and acute phase protein release were maintained or enhanced. Further, high dose TNFR:Fc, a dose similar to that associated with increased mortality in a clinical trial of sepsis (21), was unexpectedly less immunosuppressive than low dose TNFR:Fc. This loss of effect was not due to release of bound TNF from TNFR:Fc as measured by circulating TNF bioactivity and suggests the presence of a previously undescribed escape mechanism in vivo from the anti-inflammatory effects of TNFR:Fc. These data suggest that TNFR:Fc may cause imbalances in the tightly controlled inflammatory response to endotoxin. These imbalances in the context of the clinical trial may have contributed to the harmful effect of TNFR:Fc on survival from septic shock.

TNFR:Fc was a potent inhibitor of TNF cytotoxic activity and greatly increased the binding capacity of plasma for circulating TNF. In treated subjects, less than 8 ± 4 pg/ml of biologically active TNF was detected at any time during the first 24 h of the study. Furthermore, samples from 6 h or 24 h had the capacity to buffer large amounts of exogenously added rhTNF. These data show that TNFR:Fc serves both as an antagonist and as a carrier protein during systemic TNF release (10).

In TNFR:Fc subjects several factors may have accounted for the large increase in both immunoreactive TNF and the amount of biologically active TNF released from TNFR:Fc-TNF complexes after incubation with the M1 anti-TNFR Ab. The clearance of TNF was probably decreased by the carrier protein function and extended half-life of TNFR:Fc (10). Because of its high affinity for TNF, TNFR:Fc may also compete with the pool of cell surface-bound TNF and thereby increased the concentrations of immunoreactive TNF in the circulation (10). Lastly, it is unknown whether

**Table 1. Systemic hemodynamics following endotoxin and TNFR:Fc administration**

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<th>Maximum/Minimum</th>
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<td>p-value</td>
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<td>CI (L·min⁻¹·m⁻²)</td>
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<td>HR (beats/min)</td>
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<td>DO₂ (mL·min⁻¹·m⁻²)</td>
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<td>674 ± 95.1</td>
<td>923 ± 76</td>
<td>1015 ± 99.5</td>
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a Maximum or minimum for all variables occurred at 5 h after endotoxin except for MAP (8 h). The amounts of fluid administered for volume loading between 3 and 5 h were similar between the two groups (placebo, 3.6 ± 0.4 L; TNFR:Fc, 3.3 ± 0.1 L).

b Change over time in both placebo and TNFR:Fc subjects. Abbreviations and formulae: CI, cardiac index = cardiac output/body surface area; HR, heart rate; MAP, mean arterial pressure; SVRI, systemic vascular resistance index = MAP - central venous pressure/CI X 80; EF, left ventricular ejection fraction; arterial or mixed venous oxygen content (CvO₂) = [(Hb x 1.34 x % sat) + [pO₂ x 0.0031]]; VO₂, oxygen consumption = CI x (CAO₂ - CVO₂); DO₂, oxygen delivery = CI x CAO₂, O₂ER = oxygen extraction ratio = (CAO₂ - CVO₂)/CAO₂.
Changes in E-selectin (A), lactoferrin (B), and TNF receptor (p55) (C), following endotoxin administration in subjects randomized to receive placebo, low dose, or high dose TNFR:Fc. Data presented as mean and SEM and analyzed by three-way ANOVA.

An unexpected finding of the current study was that as the dose of TNFR:Fc was increased and more immunogenic TNF was detected, some of the suppressive effects on secondary cytokine and stress responses were lost, despite the continued absence of TNF plasma bioactivity. High dose TNFR:Fc was associated with an apparent escape from the suppressive effects observed with low dose TNFR:Fc; levels of cytokines and stress hormones were greater than low dose TNFR:Fc and approached levels found in the control group. The mechanism of this rebound response is not readily apparent but may involve the activation of redundant proinflammatory pathways or increased cell-associated TNF that are triggered by the neutralization of circulating TNF. This hypothesis is supported by the finding that IL-6 levels in high dose TNFR:Fc subjects were similar to those seen in the placebo subjects. IL-6 is a major counterregulatory cytokine and considered a sensitive indicator of the overall intensity of the inflammatory response in sepsis (41, 42). Conceivably, TNF antagonism may have interfered with endotoxin clearance and thus enhanced other endotoxin-dependent responses. It is unknown whether Fcγ receptors were cross-linked and activated by TNF-TNFR:Fc complexes (43). However, TNFR:Fc alone or in combination with TNF does not activate serum complement in vitro (C.-W. Vogel, unpublished data) making this an unlikely explanation. Although the mechanism of this abnormal dose response by TNFR:Fc is unknown, our
data shows that as blockade of circulating TNF activity is increased with TNFR:Fc, secondary responses escape the suppressive effect of this intervention.

In anesthetized chimpanzees given reference endotoxin (4 ng/kg, i.v.), an anti-TNF Ab (15 mg/kg) blocked TNF cytotoxicity and decreased levels of IL-6, IL-8, IL-10, lactoferrin, and elastase (44, 45). The similarities with the current study include the neutralization of circulating TNF, the suppression of the IL-6 and IL-8, and inhibition of neutrophil degranulation. However, IL-10 levels were not suppressed by either dose of TNFR:Fc. In comparing these two studies, the difference in IL-10 response may relate to species differences, the type of anti-TNF agent used, or its dose. In contrast to humans, the primates did not develop leukopenia at 1 h and their leukocytosis was almost twofold greater. Changes in the percentage of neutrophil band forms, fever, heart rate, and blood pressure responses were not reported (44, 45). Only one dose of Ab was tested in the primates and in the current study, the secondary consequences of TNF inhibition by TNFR:Fc varied with the dose. Others have postulated that the dose of the TNF inhibitor, its binding characteristics, and the clearance rate of the TNF-antagonist complex may determine whether a given agent acts as agonist or an antagonist (43). This suggests that any TNF binding agent has the potential to dysregulate the normal host response to endotoxin. For example, in primates given endotoxin and anti-TNF Ab, fibrinolysis was inhibited without altering the activation of coagulation and the authors postulate that this may predispose to microvascular thrombosis during sepsis (44).

The febrile response to endotoxin was delayed but not diminished following TNFR:Fc, despite neutralization of TNF bioactivity and suppression of IL-1 and IL-6. This suggests that other pyrogens can initiate and sustain this response or that noncirculating TNF can produce fever. MIP-1α, an endogenous pyrogen, was not affected by TNFR:Fc and may have contributed to the delayed febrile response (46). TNF inhibition significantly decreased the rate of rise to maximal temperature but did not change the duration or magnitude of fever. In contrast, the febrile response to endotoxin in rats was enhanced by TNF antagonists, suggesting important species differences in antipyretic mechanisms (47).

The hemodynamic profiles of acute endotoxemia and sepsis are similar (24). When TNF Abs are given with near-lethal doses of endotoxin to anesthetized primates, variable improvement is seen endotoxin to anesthetized primates, variable improvement is seen. Further, the correlation of immunoactive TNF with mean arterial pressure was altered by TNFR:Fc and the order of the correlation coefficients were similar (placebo > high dose > low dose) to the cytokine and stress hormone responses. This suggests that the relation of immunogenic TNF to mean arterial pressure was uncoupled by TNFR:Fc either by directly increasing immunogenic TNF because of the role of TNFR:Fc as a carrier protein or by the activation of other inflammatory mediators. Although TNF can produce the cardiovascular abnormalities associated with sepsis (50, 51), redundant non-TNF pathways activated by endotoxin or noncirculating TNF may be primarily responsible for the changes in vascular tone, heart rate, oxygen consumption, and cardiac function during the early acute phase response.

This study demonstrates the complex and unexpected interactions that may occur following inhibition of a single arm of the inflammatory response and in conjunction with the clinical trial of TNFR:Fc, underscores the potential for such approaches in septic shock to be ineffective or harmful (2, 20–23). The current study suggests that failure of these therapies in septic shock could relate to redundant TNF-independent inflammatory pathways, impairment of protective host inflammatory responses, or disruption of the normal sequence of immune activation during infection. Inhibition of TNF was not associated with any improvement in clinical manifestations, particularly the cardiovascular responses that play a major role in the mortality of sepsis (2). In sum, these data demonstrate that inflammatory responses are not uniformly reduced but rather may be dysregulated by TNFR:Fc. However, these results do not exclude the possibility that TNF antagonists may have beneficial effects in noninfectious inflammatory diseases such as rheumatoid arthritis (52, 53).

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