Pretreatment with a 55-kDA tumor necrosis factor receptor-immunoglobulin fusion protein attenuates activation of coagulation, but not of fibrinolysis during lethal bacteremia in baboons


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Pretreatment with a 55-kDa Tumor Necrosis Factor Receptor–Immunoglobulin Fusion on Proteins Attenuates Activation of Coagulation, but not of Fibrinolysis, during Lethal Bacteremia in Baboons


Baboons (Pap o anubis) were injected with a lethal intravenous inoculum of live Escherichia coli (O86:B7) and pretreated with either a 55-kDa tumor necrosis factor (TNF) receptor–IgG fusion protein (TNFR55:IgG) (n = 4, 4.6 mg/kg) or placebo (n = 4). Neutralization of TNF activity by TNFR55:IgG treatment in baboons was associated with complete prevention of mortality and a strong attenuation of coagulation activation reflected by the plasma concentrations of thrombin–antithrombin III complexes (P < .05). Act activation of fibrinolysis was not influenced by TNFR55:IgG (plasma tissue-type plasminogen activator (P < .05)). Furthermore, TNFR55:IgG modestly reduced release of secretory phospholipase A2. These data suggest that endogenous TNF contributes to activation of coagulation, but not to activation of fibrinolysis during severe bacteremia.

Tumor necrosis factor-α (TNF) is considered to be a key mediator in the pathogenesis of sepsis syndrome. TNF is released into the circulation early after intravenous bacterial challenge in animals, and neutralization of endogenous TNF prevents lethality in these acute models [1]. The role of TNF in disturbances of the hemostatic mechanism during systemic infection is less clear. Sepsis- and endotoxin-induced activation of the coagulation system is driven by the tissue factor–mediated extrinsic pathway [2]. TNF potently up-regulates tissue factor expression on endothelial and mononuclear cells, suggesting a role for this cytokine in coagulation activation [1, 2]. In accord, intravenous injection of recombinant TNF into humans or baboons induced activation of the common pathway of the coagulation system [3, 4]. However, treatment of baboons with severe bacteremia or endotoxemia with a neutralizing anti-TNF monoclonal antibody did not result in a noticeable effect on activation of the coagulation system, in spite of the fact that such treatment afforded significant protection against mortality [5–7]. Similarly, anti-TNF did not influence coagulation activation during nonlethal endotoxemia in chimpanzees [8]. By contrast, anti-TNF completely prevented the fibrinolytic response to nonlethal endotoxemia [8], whereas in the lethal models fibrinolysis was not investigated.

Recently, the capacity of a 55-kDa TNF receptor–IgG fusion protein (TNFR55:IgG) to effectively neutralize TNF activity and to strongly reduce lethality in baboons infused with a lethal dose of live Escherichia coli was reported [9]. Interestingly, treatment with TNFR55:IgG attenuated fibrinogen consumption and the increases in prothrombin and partial thromboplastin times caused by the bacteremia. These findings prompted us to study in more detail the effect of TNF:IgG on the activation of coagulation and fibrinolysis.

Methods

Study design. The present study was performed simultaneously with a previously reported investigation [9]. Details of the animal study have been reported elsewhere [9]. Briefly, baboons (Pap o anubis) (10–14 kg) were challenged with E. coli with or without pretreatment with TNFR55:IgG at the Research Animal Resource Center of Cornell University Medical College. At time zero, all animals received 103–104 cfu/kg live E. coli (O86:B7) through a femoral venous catheter over 30 min. Baboons were randomized to receive either TNFR55:IgG (Ro 45-2081; 4.6 mg/kg; n = 4) or placebo (n = 4) as a 15-min intravenous infusion directly prior to infusion of bacteria. Endotoxin concentration of the TNFR55:IgG preparation was <0.4 EU/mg of protein. Arterial blood was obtained at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 h relative to the infusion of E. coli.
Assays. All assays were performed in heparinized plasma samples and were described in detail previously [4, 10]. Coagulation activation was determined by measuring thrombin–antithrombin III (TAT) complexes by ELISA. Fibrinolytic activation was monitored by measurements of tissue type plasminogen activator (tPA) by ELISA, plasminogen activator inhibitor type 1 (PAI-1) by ELISA, and plasmin–α2-antiplasmin (PAP) complexes by RIA. Levels of PAP complexes are expressed as percentage of the level present in normal baboon plasma in which a maximal amount of PAP complexes was generated by a 1-h incubation with an equal volume of urokinase (50 μg/mL) in the presence of 0.4 M methylamine. Neutrophil degranulation was determined by measurement of the plasma concentrations of elastase–α1-antitrypsin complexes by RIA. Secretory phospholipase A2 (sPLA2) was measured with an ELISA.

Statistical analysis. All values are expressed as mean ± SE. Differences within groups were tested by repeated-measures analysis of variance. Differences between groups were tested by repeated-measures analysis of variance (interaction between treatment and time). P values are therefore derived from analyses in which data from all time points were included. P < .05 was considered to represent a significant difference.

Results

In the previously reported study, treatment with TNFR55:IgG had been found to completely neutralize TNF activity throughout the observation period and to significantly attenuate the severe hypotensive response and lethality observed in the animals infused with E. coli [9]. Three of four baboons pretreated with placebo had died, after 6, 30, and 36 h, respectively, while all baboons treated with TNFR55:IgG survived.

The infusion of E. coli was associated with a strong activation of the common pathway of the coagulation system, as reflected by a sustained rise in the plasma concentrations of TAT complexes, peaking after 6 h (959 ± 296 ng/mL; P < .05 vs. time) (figure 1). This coagulation response was significantly blunted by treatment with TNFR55:IgG. Peak levels of TAT complexes in TNFR55:IgG-treated animals were 322 ± 90 ng/mL (P < .05 vs. placebo). Bacteremia also induced a marked activation of the fibrinolytic system, as indicated by increases in the plasma concentrations of tPA (peak of 12.9 ± 2.2 ng/mL after 8 h; P < .05 vs. time), PAI-1 (peak of 3669 ± 1298 ng/mL after 8 h; P < .05), and PAP complexes (peak of 0.68% ± 0.32% after 4 h; P < .05) (figure 1). Treatment with TNFR55:IgG had no influence on the rise in tPA levels but significantly attenuated the release of PAI-1 during bacteremia. Peak PAI-1 levels in TNFR55:IgG-infused baboons were 1095 ± 314 ng/mL (P < .05 vs. placebo). The plasma concentrations of PAP complexes tended to be higher in TNFR55:IgG-treated animals (peak of 0.85% ± 0.32% after 4 h), but the difference compared with control animals did not reach statistical significance.

As reported earlier [9], bacteremia was associated with a sustained neutrocytopenia, which was partially reversed by

Figure 1. Effect of pretreatment with 55-kDa TNF receptor–IgG fusion protein (TNFR55:IgG) on activation of coagulation and fibrinolysis. Data are mean (±SE) plasma concentrations of thrombin-antithrombin III (TAT) complexes, tissue-type plasminogen activator (tPA), plasminogen activator inhibitor type 1 (PAI-1), and plasmin–α2-antiplasmin (PAP) complexes after infusion of Escherichia coli in baboons pretreated with either TNFR55:IgG (n = 4, ●) or placebo (n = 4, ○). P indicates difference between groups by analysis of variance. NS = nonsignificant.
protein results in an attenuated procoagulant response, as re-
flected by inhibition of release of TAT complexes, in the ab-

ence of an effect on activation of fibrinolysis. This result is
in line with the previously reported inhibition of more rough
measures of coagulation activation, such as fibrinogen con-
sumption and prolongation of prothrombin and partial thrombo-

plastin times [9]. However, it should be noted that, thus far,
studies in which endogenous TNF was neutralized in primates
with either lethal or sublethal bacteremia or endotoxemia did
not reveal any effect on activation of the coagulation system
[5–8], while anti-TNF did abrogate the fibrinolytic response
to low-dose endotoxin in chimpanzees [8]. We do not have an
explanation for the apparent discrepancy of the present results
with those from earlier studies, other than that the compound
used to neutralize TNF activity, a dimeric 55-kDa TNF recep-
tor–IgG fusion protein (which also has affinity for lympho-
toxin), was different.

We consider it unlikely that our results are influenced by
less-than-complete neutralization of TNF. Plasma TNF activity
remained completely neutralized (as determined by the highly
sensitive WEHI cytotoxicity assay) in animals treated with
TNFR55:IgG throughout the entire observation period [9]. In
fact, even lower doses of TNFR55:IgG than used in this study
were able to completely neutralize TNF activity [9]. Further,
TNFR55:IgG has been found to form stable complexes with
TNF, in which aspect it differs from a similar IgG fusion
protein containing a dimeric p75 TNF receptor [11]. Further,

Figure 2. Effect of 55-kDa TNF receptor–IgG fusion protein
(TNFR55:IgG) on neutrophil degranulation and secretory phospholi-
pase A2 (sPLA2) release. Data are mean (±SE) plasma concentra-
tions of elastase–α1-antitrypsin complexes and sPLA2 after infusion of
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TNFR55:IgG. Infusion of E. coli also resulted in degranulation
of neutrophilic granulocytes, as reflected by an increase in the
plasma levels of elastase–α1-antitrypsin complexes, maximal
levels being measured at the end of the study period at 8 h
(1234 ± 153 ng/mL; P < .05 vs. time) (figure 2). TNFR55:IgG
significantly inhibited this response, with elastase–α1-antitr-
ypsin complexes reaching an approximately constant level at
~500 ng/mL from 1 to 8 h (P < .05 vs. placebo). E. coli
bacteremia further caused a rise in the plasma concentrations
of sPLA2, the highest levels being measured at the end of the
observation period (8 h: 4217 ± 2081 ng/mL; P < .05 vs.
time) (figure 2). Although TNFR55:IgG reduced sPLA2 release
(8 h: 1845 ± 1284 ng/mL), the difference with control baboons
did not reach statistical significance because of a large interindi-
vidual variation.

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The main finding of this study is that pretreatment of baboons
with severe E. coli bacteremia with a TNF receptor–IgG fusion

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One control animal had died at the time the last blood sample was taken (after 8 h). The lack of this sample is unlikely to influence the results, since plasma concentrations of PAI-1, elastase–α1-antitrypsin complexes, and sPLA2 were higher in more ill animals, and thus the availability of an 8-h sample from the baboon that died early would likely have made differences between control animals and animals treated with TNFR55:IgG more pronounced.

Severe *E. coli* bacteremia in baboons may be a useful model to study pathogenetic mechanisms underlying inflammatory responses during fulminant septic shock. We herein demonstrate that neutralization of endogenous TNF by pretreatment with a 55-kDa TNF receptor–IgG fusion protein not only prevents lethality but also significantly attenuates coagulation activation while not influencing fibrinolysis. Compared with earlier findings in primates with mild endotoxemia, revealing unaltered coagulation activation and inhibited fibrinolytic activation in animals treated with an anti-TNF antibody [8], these data illustrate the divergence of various sepsis models, which depend on the severity and the time course of toxicity evoked by the bacterial insult, and also point to the difference in the effects exerted by the various anti-TNF agents used in such studies.

References