Effects of 2 Different Anti–Tumor Necrosis Factor–α Agents in a Primate Model of Subcutaneous Abscess Formation

Xiao-yu R. Song,1 Floyd Fox,2 Mary Ann Gallo,3 Amy Rosenberg,1 Robert Jordan,1 David Shealy,1 Xiao-yu R. Song, 1 Floyd Fox, 2 Mary Ann Gallo, 3 and Carrie Wagner 1

1Research and Development, Centocor, Malvern, Pennsylvania; 2ImClone Systems, Somerville, New Jersey; 3Primedica Corporation, Worcester, Massachusetts

Tumor necrosis factor (TNF–α) exerts both physiologic and pathologic effects in response to infection, conferring the benefit of host defense against infection at the risk of eliciting severe pathology if the response is excessive or inappropriate. In the present study, the effects of an anti–TNF–α monoclonal antibody (MAb) and a TNF–α receptor construct (p75-Fc) were compared with that of saline in a primate model of subcutaneous abscess induced with Staphylococcus aureus. Intravenous administration of anti–TNF–α MAb delayed the onset and reduced the incidence and the severity of abscess formation in response to inoculation with S. aureus at concentrations of 109 and 1010 cfu/mL, compared with administration of saline. In contrast, no improvement in abscess formation was observed in animals treated with p75-Fc. These results supply initial evidence that anti–TNF–α MAb, unlike p75-Fc, provides a beneficial effect in this abscess model.

TNF–α was originally described as an endotoxin-induced, macrophage-derived factor that promotes hemorrhagic necrosis of solid tumors and the cachexia of chronic infections [1]. TNF–α exists in 2 active forms: the cell surface–associated 26-kDa prepeptide (transmembrane TNF–α; tmTNF–α) and the cleavage product, the 17-kDa secreted protein (soluble TNF–α) generated by TNF–α–converting enzyme [2]. TNF–α mediates its biological effects through 2 receptors, designated TNF–α receptor I (TNFRI; p55 receptor) and TNFRII (p75 receptor).

Since 1980, TNF–α has also been implicated in a variety of inflammatory, infectious, and malignant disorders. At the cellular level, TNF–α modulates a broad spectrum of responses, including inflammation, immunoregulation, proliferation, apoptosis, and antiviral activity [3]. TNF–α acts to strengthen host defenses against a variety of pathogenic microbes, including fungi [4], intracellular bacteria [5], and parasites [6], by promoting a broad range of immunological and inflammatory responses. However, certain aspects of the normal inflammatory response to serious infection may be detrimental to the host, as is exemplified by the septic shock that occurs after systemic release of large amounts of TNF–α. Administration of TNF–α can mimic the changes observed in individuals with septic shock. Moreover, when endogenous TNF–α is neutralized in mice, the animals can tolerate a lethal dose of lipopolysaccharide [7, 8]. Along the same lines, administration of anti–TNF–α antibodies has been shown to exert a protective effect in animal models of systemic bacteremia and endotoxemia [7–11].

TNF–α also plays a pivotal role in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA) [12, 13]. Two different anti-TNF therapeutic strategies are approved for treatment of RA. One strategy involves use of a monoclonal antibody (MAb), infliximab, to specifically bind and block the effects of human TNF–α [14]. The other approach involves use of etanercept, a soluble, genetically engineered form of 2 extracellular domains of human p75 TNFR1 linked by the Fc fragment of an IgG1 molecule [15]. One of the differences between the 2 TNF antagonists is that etanercept binds to both lymphotixin–α (LT–α) and TNF–α, whereas infliximab binds to TNF–α only. In addition, infliximab forms a more stable anti–TNF–α antibody–TNF–α complex (B. Scallon, A. Cai, N. Solowski, X.-y. Song, D. Shealy, and C. Wagner [Scallon et al., unpublished data]). LT–α, which is also known as “TNF–β,” is a member of the TNF superfamily and shares ~30% homology with TNF–α in amino acid sequence [16]. Like TNF–α, LT–α exists in soluble and transmembrane forms: the homotrimeric form (LT–α3), which binds to the same receptors as TNF–α, and a newly identified herpesvirus entry mediator receptor, the membrane-associated heterotrimeric form (LT–α1β2 or LT–α2β1) [17], which binds to its own receptor, LT–βR [18, 19]. Functionally, LT–α participates in inflammatory responses and host defense against infection, as well as in normal immune system development [20].

Because the use of etanercept in patients with sepsis resulted in a dose-dependent increase in mortality, and no profound improvement was observed when infliximab was used in patients with severe sepsis [21, 22], we decided to explore whether the approved anti-TNF therapies would similarly affect the innate...
response to infection. In the present study, we examined the effects of treatment with etanercept and with an anti–TNF-α MAb in a model of subcutaneous abscess formation induced with Staphylococcus aureus in cynomolgus monkeys. In this model, abscesses can be routinely and reproducibly induced by subcutaneous injection of S. aureus [23, 24]. The 2 anti–TNF-α therapies were initiated before bacterial challenge, and their effects on the incidence and severity of abscess formation were compared with the effects of saline treatment.

Materials and Methods

Animals. Cynomolgus monkeys (Macaca fascicularis) were purchased from Primate Products and Covance Research Products and randomly assigned to 3 treatment groups by body weight. Each group contained 2 male and 2 female monkeys. All animals were housed and the entire experiment was conducted at Primedica, using good laboratory practices (as described by the US Food and Drug Administration).

Treatment and delivery regimen. Etanercept (Embre; Immunex) was purchased from a pharmaceutical supplier. Because infliximab does not bind to monkey TNF-α, a cell line for a murine anti-human TNF-α MAb (IgG2a) that binds and neutralizes both human and monkey TNF-α was obtained from N. Voitenok (Foundation for Fundamental Research of the Republic of Belarus, Minsk, Belarus) [25]. The antibody was then produced and purified at Centocor. The vehicle control, 0.9% sodium chloride (Abbott Laboratories), was obtained from Medical Specialties; a 3 mg/kg dose of etanercept or anti–TNF-α MAb was chosen, on the basis of clinical and preclinical data that demonstrated sufficient TNF-α blocking activity without toxicity at this dose [26, 27]. Etanercept, anti–TNF-α MAb, or saline was given intravenously to 3 groups of animals on days –7, –3, 1 (day 1 was the day of bacterial challenge), and 4. Dose volumes were maintained at 0.6 mL/kg. Etanercept was administered within ~1 h of formulation.

Preparation of bacteria. One day (day −1) before bacterial challenge, 1 aliquot of frozen stock of S. aureus (ATCC 25923) was thawed and incubated in trypticase soy broth (TSB) at 37°C overnight. On the next day (day 1), aliquots of the overnight culture were added to 10-mL cultures of TSB and incubated at 37°C for 6 h. The bacterial suspensions were harvested and suspended in sterile saline at a concentration of 10^6 cfu/mL, as measured by spectrophotometric light absorbance at 530 nm. Serial dilutions were made in saline to obtain bacterial concentrations of 10^7, 10^6, 10^5, and 10^4 cfu/mL for subsequent inoculation.

Induction and monitoring of abscess formation. On day 1, ~2 h after the administration of the treatment agents, groups of cynomolgus monkeys were shaved to expose a large area of dorsal skin. Eight areas were marked with indelible ink in 2 parallel rows. Two subcutaneous injections of each concentration of S. aureus at 10^7, 10^6, 10^5, and 10^4 cfu/mL were given at the paired sites, with a 23-gauge needle, from cranial to caudal side with injection volumes of 1 mL/site. Cefazolin (20–25 mg/kg) was administered intramuscularly to each animal before bacterial challenge and approximately every 8–10 h after challenge for 72 h, to prevent bacteremia. Clinical observations were made and recorded once daily, ~2 h after treatment, on days −7, −3, 4, and 5. These observations included changes in the skin and hair, eyes and mucous membranes, respiratory system, circulatory system, central nervous system, somatomotor activity, and behavioral patterns. Bacterial injection site observations were made once daily for swelling, erythema, ulceration, and hemorrhage that had onset after the bacterial challenge. Cumulative swelling scores for each treatment group were determined by adding the scores of each bacterial injection site for a given inoculum size and a given time point; individual scores were assigned on a scale of 0–3, representing no, mild, moderate, and marked swelling. Animals were euthanized on day 8, although this type of skin infection model is usually monitored for 5–7 days. Photographs of the backs of animals were taken in situ before necropsy was performed, and the internal abscess areas were outlined and photographed again for morphometric determination of abscess surface area. The morphometric analysis was performed in a blinded fashion.

Cytokine assays. Serum samples were separated from whole blood, which was collected from a peripheral vein before drug delivery on days −7, −3, 1, 3, and 4. Serum levels of interleukin (IL)–1β, interferon (IFN)–γ, and IL–8 were determined by ELISA at dilutions of 1:10 for IL–12, 1:4 for IFN–γ and IL–8, and 1:5 for cell culture supernatants, according to the manufacturer’s instructions (Biosource International).
that contained cyno TNF-α were preincubated for 30 min with a 1:400 dilution of cell supernatant that contained cyno TNF-α and then added to WEHI cells, as described in Materials and Methods. Cell viability, as reflected by absorbance at 550–650 nm, was high in the absence of TNF-α, which was comparable to that seen in the absence of monkey TNF-α (figure 1A). In contrast, when anti–TNF-α MAb and etanercept were examined for neutralization of monkey or human LT-α, only etanercept suppressed the cytotoxicity of monkey LT-α, whereas anti–TNF-α MAb showed no effect on monkey LT-α activity, as reflected by low cell viability similar to that seen with the LT-α control (figure 1B). These findings further demonstrated that etanercept blocked both TNF-α and LT-α activities, whereas anti–TNF-α MAb blocked only TNF-α bioactivity [19]. Moreover, etanercept neutralized monkey TNF-α more efficiently than did anti–TNF-α MAb, with a 50-fold difference in IC_{50} in this assay (figure 1A).

Impact of anti–TNF-α MAb, but not etanercept, on S. aureus-induced abscess formation in cynomolgus monkeys. To assess the effect of different anti–TNF-α therapies on bacterial infection in vivo, we used an established animal model of subcutaneous abscess formation. To assess whether ongoing anti–TNF-α therapies could lead to increased susceptibility to cutaneous infection in this model, saline, anti–TNF-α MAb, and etanercept were delivered intravenously to 3 groups of cynomolgus monkeys, at 3 mg/kg, with a dose volume of 0.6 mL/kg, at days 7, -3, 1 (2 h before inoculation with S. aureus), and 3. Differences among treatment groups were observed at sites at which 10^9 or 10^10 cfu/mL of S. aureus was injected subcutaneously. First, a

Statistical analysis. All comparisons were made between groups treated with anti–TNF-α MAb or with etanercept and the saline-treated control group. Intergroup differences were assessed for statistical significance by the Mann-Whitney U test, for abscess area; by the 2-tailed t test, for cytokine levels; or by area under the curve of cumulative swelling scores, for abscess formation. P < .05 was considered to be statistically significant.

Results

In vitro binding characteristics of anti–TNF-α MAb and etanercept. To examine the ability of etanercept and of anti–TNF-α MAb to neutralize TNF-α and/or LT-α, we used an in vitro cytotoxicity assay. Both anti–TNF-α MAb and etanercept inhibited the cytotoxicity of monkey TNF-α in a bioassay that used WEHI cells, as reflected by high cell viability, which was comparable to that seen in the absence of monkey TNF-α (figure 1A). In contrast, when anti–TNF-α MAb and etanercept were examined for neutralization of monkey or human LT-α, only etanercept suppressed the cytotoxicity of monkey LT-α, whereas anti–TNF-α MAb showed no effect on monkey LT-α activity, as reflected by low cell viability similar to that seen with the LT-α control (figure 1B). These findings further demonstrated that etanercept blocked both TNF-α and LT-α activities, whereas anti–TNF-α MAb blocked only TNF-α bioactivity [19]. Moreover, etanercept neutralized monkey TNF-α more efficiently than did anti–TNF-α MAb, with a 50-fold difference in IC_{50} in this assay (figure 1A).

Intravenous inoculation of 10^9 or 10^10 cfu/mL of S. aureus led to the formation of one or more abscesses in all experimental animals. The sizes of these abscesses were measured over time by 2 methods: (1) by the 2-tailed t test, for cytokine levels; or by area under the curve of cumulative swelling scores, for abscess formation. P < .05 was considered to be statistically significant.

Table 1. Incidence of abscess formation in cynomolgus monkeys treated with anti–tumor necrosis factor (TNF–α), monoclonal antibody (MAb), etanercept, or saline and inoculated with Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Bacterial concentration</th>
<th>Incidence of abscess formation (%)</th>
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<td>With saline (n=4)</td>
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<td></td>
<td>Day 3</td>
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<tr>
<td>10^9 cfu/mL</td>
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<td>10^10 cfu/mL</td>
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NOTE. Incidence of abscess formation is expressed as the percentage of the total injection sites in each treatment group that had erythema and/or swelling.
noticeable delay in the onset and a reduction in the incidence of abscess formation after bacterial challenge with 10^9 cfu/mL were detected in the group of monkeys treated with anti–TNF-α MAb, compared with the saline-treated animals (table 1). Moreover, severity of the abscess formation at individual inoculation sites, as measured by both swelling and erythema, was markedly less as early as day 3 and through day 4 in animals treated with anti–TNF-α MAb than it was in saline-treated animals (table 2). In contrast, etanercept treatment resulted in no improvement and in some cases a worsening in swelling and erythema, assessed qualitatively, at local inoculation sites on day 3 and day 4, compared with the results of saline treatment (table 2). A modest increase in the incidence of abscess formation after challenge with 10^9 cfu/mL was also observed in the etanercept-treated group on day 3, compared with that in the saline-treated group (75% vs. 50%). When the changes in swelling at all inoculation sites for all animals were compared quantitatively among different treatment groups, a statistically significant reduction in cumulative swelling score was detected only in the anti–TNF-α MAb–treated group, com-

Table 2. Severity of abscess formation at individual injection sites in cynomolgus monkeys treated with anti–tumor necrosis factor (TNF)–α monoclonal antibody (MAb), etanercept, or saline and inoculated with Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Bacterial concentration, sex of animal, day</th>
<th>Saline</th>
<th>Etanercept</th>
<th>Anti–TNF-α MAb</th>
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<td>10^9 cfu/mL, Male</td>
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<td>10^10 cfu/mL, Female</td>
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<td>10^10 cfu/mL, Female</td>
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NOTE. Bacterial challenge was done on day 1. The degree of swelling was described as none (-), mild (+), or moderate (++). E, erythema.

opened early abscess formation, compared with 50% of the saline-treated animals (table 1). Moreover, severity of the abscess formation at individual inoculation sites, as measured by both swelling and erythema, was markedly less as early as day 3 and through day 4 in animals treated with anti–TNF-α MAb than it was in saline-treated animals (table 2). In contrast, etanercept treatment resulted in no improvement and in some cases a worsening in swelling and erythema, assessed qualitatively, at local inoculation sites on day 3 and day 4, compared with the results of saline treatment (table 2). A modest increase in the incidence of abscess formation after challenge with 10^9 cfu/mL was also observed in the etanercept-treated group on day 3, compared with that in the saline-treated group (75% vs. 50%). When the changes in swelling at all inoculation sites for all animals were compared quantitatively among different treatment groups, a statistically significant reduction in cumulative swelling score was detected only in the anti–TNF-α MAb–treated group, com-

Figure 2. Suppressive effect of anti–tumor necrosis factor (TNF)–α monoclonal antibody (MAb) on cumulative swelling scores. The cumulative swelling score was the sum of the swelling score for each individual injection site at a certain time point for each treatment group, after inoculation with Staphylococcus aureus at a concentration of either 10^9 (A) or 10^10 (B) cfu/mL. The areas under the curve for the anti–TNF-α MAb–treated group and the etanercept-treated group were compared with that of the saline-treated group by use of the t test. *P < .005, vs. saline control.
pared with the score of the saline-treated group, at both doses (figure 2A and 2B; P < .005). Although no obvious abscesses were observed in different treatment groups at the inoculation sites when 10^7 cfu/mL was injected subcutaneously, when 10^8 cfu/mL was used, the incidence of abscess formation in etanercept-treated group was 1 of 8, compared with 0 of 8 in both saline- and anti–TNF-α MAb–treated groups, on day 4 (data not shown).

Finally, on close examination of the bacterial injection sites on day 8, more pronounced swelling, erythema, and hemorrhage were observed in etanercept-treated animals than in saline-treated animals (figure 3). However, when median abscess area for 8 injection sites within each treatment group was quantified on day 8, no significant difference in the median abscess area was detected between etanercept-treated animals and saline-treated animals (figure 4). In contrast, a significant reduction in the median abscess area was detected in the animals treated with anti–TNF-α MAb, compared with that of saline-treated animals, after challenge with 10^9 cfu/mL (figure 4).

**In vivo neutralizing abilities of anti–TNF-α MAb and etanercept.** To further examine whether the effect of anti–TNF-α MAb was due to its specific binding and subsequent blocking of TNF-α activity, we first tested whether any TNF-α was present in the serum samples obtained from different treatment groups. Because no cytotoxicity was detected in serum samples, even in those from the saline-treated group (data not shown), 1 ng/mL of TNF-α or LT-α was spiked in before the samples were added to the WEHI cells. The serum TNF-α neutralization ability reached peak levels on day −3 and was sustained through day 3 in animals treated with anti–TNF-α MAb (figure 5A). In contrast, no LT-α neutralization was detected in serum samples from animals treated with anti–TNF-α MAb (figure 5B). Finally, neutralization of both TNF-α (figure 5A) and LT-α activities (figure 5B) was detected as early as day −3 in serum samples from etanercept-treated animals, whereas no neutralization of either TNF-α or LT-α activity was detected in samples from saline-treated animals (data not shown). Therefore, functionally active anti–TNF-α MAb and etanercept were present in the serum of animals treated with these agents.

**Systemic effects of anti–TNF-α MAb and etanercept.** To determine whether the impact of anti–TNF-α MAb was localized to the inoculation site or manifested systemically, we monitored the peripheral white blood cell count, hematology profile, body temperature, and body weight of animals in all 3 treatment groups. No significant differences were found in these parameters among treatment groups (data not shown).

**Immune response against anti–TNF-α MAb and etanercept.** We next sought to examine the presence of antibodies against the active drugs in the serum samples of the treated animals to better evaluate the effects of these therapies. There are 2 reasons for examining the potential immune response. First, either of these agents might be immunogenic in monkeys, because the antibody used for the study is a murine anti–human TNF-α antibody, and etanercept is a human p75 TNF-α receptor:IgG Fc
fusion protein. Second, both agents were delivered 4 times to the experimental monkeys over a 15-day study period. An immune response against anti–TNF-α MAb was detected, which reached a peak on day 4 and was sustained until day 8. An immune response against etanercept also was detected, as early as day 3, and increased over time (figure 6). However, the detected immune response had no apparent effect on the neutralizing capacity of the serum (figure 5).

Effect of anti–TNF-α MAb on circulating IL-12 and IL-8. To further understand the underlying mechanisms by which anti–TNF-α MAb suppressed subcutaneous abscess formation, the levels of inflammatory mediators known to be involved in innate response to bacterial challenge were examined [29]. First, IL-12, which was detected in the circulation of the bacteria-challenged animals, was moderately suppressed (nearly 2-fold) by anti–TNF-α MAb treatment on both day 3 (figure 7A) and day 4 (data not shown). In contrast, a slight elevation, albeit statistically insignificant, of IL-12 was detected in etanercept-treated animals on day 3 (figure 7A) and day 4 (data not shown), whereas circulating levels of IFN-γ, another key inflammatory cytokine involved in innate immune response that is regulated by IL-12, were very low—therefore, no meaningful comparison could be made (data not shown). We also measured serum IL-8 levels in these animals, because IL-8 plays a crucial pathologic role in various inflammatory diseases and is regulated by TNF-α [30, 31]. Anti–TNF-α MAb significantly suppressed the production of IL-8 on day 8 (figure 7B), which became evident as early as day 4 (data not shown). In contrast, etanercept did not alter the serum level of IL-8 significantly (figure 7B).

Suppressive effects of anti–TNF-α MAb on IL-12 production from PBMC. Because IL-12 is largely produced by macrophages and B cells, we next examined whether anti–TNF-α MAb directly or indirectly affected IL-12 production from PBMC in response to TSST-1 in vitro. TSST-1, a staphylococcal exotoxin, is the most important disease-associated superantigen and has been shown to elicit the release of predominantly Th1-type cytokines, including IL-12, IFN-γ, and LT-α [29, 32, 33]. PBMC (2 x 10^5 cells/well) isolated from healthy human donors were cultured for 68 h in the presence or absence of 200 ng/mL of...
TSST-1, TSST-1 with anti–TNF-α MAb, or TSST-1 with etanercept, and supernatants were tested for IL-12 levels. The baseline level of IL-12 was below the detection limit, but the addition of TSST-1 increased IL-12 production in control cells. This increase was moderately curtailed, though not by a statistically significant amount, by both anti–TNF-α MAb and etanercept treatment (figure 8).

**Discussion**

In the present study, we have shown that administration of anti–TNF-α MAb to *S. aureus*–challenged cynomolgus monkeys results in both a delay in the onset and a reduction in the incidence and severity of abscess formation, compared with administration of saline. Anti–TNF-α MAb appears to suppress the circulating levels of IL-12 and IL-8 in response to *S. aureus* challenge. Interestingly, delivery of etanercept, another anti–TNF-α agent, using the same regimen resulted in a modest increase in the incidence of abscess formation and no improvement in the severity of abscess formation, compared with treatment with saline.

TNF-α exerts both physiologic and pathologic effects in response to infection and is considered to be a double-edged sword, conferring the benefit of host defense against infection at the risk of severe pathology if the response is excessive or inappropriate. The pathologic effects of TNF-α may lead to organ dysfunction and death, as occurs in patients with septic shock [34]. The beneficial effects of anti–TNF-α MAb, as shown in the present study, indicate that the efficacy may be derived, in part, from its ability to interrupt the cycle of TNF-α production that results from macrophage stimulation on encountering bacteria, via an autocrine mechanism. We have demonstrated that serum samples obtained from animals treated with anti–TNF-α MAb exhibited strong neutralizing ability toward bioactive TNF-α, which suggests that sustainable amounts of antibody are present in the circulation that likely account for the blockade of excessive production of this otherwise injurious molecule.

Although IL-12 exerts a protective effect in viral, parasitic, or intracellular bacterial pathogen–induced infections, the role of IL-12 in the host response to gram-positive bacteria is not clear. Administration of IL-12 before, but not after, infection can protect mice from a lethal *S. aureus* infection [35]. Our finding that the IL-12 level was moderately decreased on days 3 and 4 in animals treated with anti–TNF-α MAb led us to hypothesize that anti–TNF-α MAb could exert a protective effect against severe abscess formation via suppression of IL-12 production after bacterial challenge. IL-12 is known to activate macrophages and NK cells and to induce production of cytokines such as IFN-γ and IL-12.

**Figure 6.** Immune response in cynomolgus monkeys against anti–tumor necrosis factor (TNF)–α monoclonal antibody (MAb) or etanercept. Serum levels of antibodies against etanercept (triangles) or anti–TNF-α MAb (squares) at indicated times were determined as described in Materials and Methods and are expressed as mean ± SE of optical density readings at 492 nm.

**Figure 7.** Suppression of circulating interleukin (IL)–12 and IL-8 by anti–tumor necrosis factor (TNF)–α monoclonal antibody (MAb) treatment. Serum levels of IL-12 (A) and IL-8 (B) were determined by ELISA in serum samples obtained on days 3 (A) and 8 (B) after bacterial challenge of cynomolgus monkeys treated with anti–TNF-α MAb, etanercept, or saline. Data are mean ± SE of results from each treatment group. *P < .05, vs. saline control.
Figure 8. Effect of anti–tumor necrosis factor (TNF)–α monoclonal antibody (MAB) and etanercept on peripheral blood mononuclear cells (PBMC) in response to toxic shock syndrome toxin (TSST)–1. PBMC isolated from 3 independent healthy human donors were incubated with 200 ng/mL of TSST-1 in normal culture medium in the absence and in the presence of 10 μg/mL of etanercept or anti–TNF-α MAB. Cell culture supernatants were collected 68 h after incubation and assayed for interleukin (IL)–12 at a 1:5 dilution. Bars show the mean ± SE of results from duplicate cultures. Data are from 3 experiments.

TNF-α [36]. The modest suppression of IL-12 by anti–TNF-α MAB likely could provide an additional regulatory mechanism to keep macrophage activation and subsequent TNF-α production in check. In addition, IL-12 mediates the bacterial toxin–induced expression of a lymphocyte homing molecule, cutaneous lymphocyte-associated antigen (CLA), on T cells, thus contributing to skin rashes associated with superantigen-mediated disease [37–40]. Local IL-12 production in combination with TNF-α and IL-1 would induce the expression of E-selectin on vascular endothelium, allowing an initial influx of CLA-positive effector cells activated by superantigen. This could favor the progression of the staphylococcal skin colonization. However, if IL-12 is produced in a similar fashion in systemic infections, it is likely that generalized T cell activation with concomitant CLA induction could lead to diffuse extravasation of activated T cells at the infection site, particularly the skin, resulting in the release and spillover of a host of inflammatory mediators and, subsequently, widespread organ damage. Our in vitro data are complementary to our in vivo observations; anti–TNF-α MAB treatment dampened the ability of TSST-1–stimulated PBMC to produce IL-12. Therefore, the moderate inhibition of IL-12 production by anti–TNF-α MAB likely has multiple downstream consequences, including reductions in swelling, erythema, abscess area, and the local inflammatory response at the injection sites, as observed on days 3, 4, and 8, and providing a mechanism for prevention of a widespread systemic infection.

The novel demonstration of the different effects of the 2 anti–TNF-α therapeutic agents, anti–TNF-α MAB and etanercept, on abscess formation in this nonhuman primate model provides additional insight into the differential immunoregulatory effects of these 2 therapeutic modalities. Recent gene-targeting studies have demonstrated that LT-α plays a very important role in lymphoid organogenesis [20]. Because secondary lymphoid organs such as the spleen, lymph nodes, and Peyer’s patches are the sites at which immune cells interact and elicit immune response against foreign antigens [41], transient blockade of LT-α could interfere with normal immune defense mechanisms. This was supported by our finding that etanercept treatment led to no improvement in cumulative swelling scores or abscess area, compared with saline treatment (figures 2 and 3).

Alternatively, the different effects on abscess formation observed when anti–TNF-α MAB and etanercept were compared with saline treatment might result from differences in the magnitude of the TNF-α blocking effects. Anti–TNF-α antibody binds and neutralizes TNF-α with high affinity and specificity [14] and can form an anti–TNF-α antibody–TNF-α complex that is much more stable than the TNF-α–p75-Fc construct complex (Scallon et al., unpublished data). In addition, anti–TNF-α antibody binds not only to the trimeric form of tmTNF-α but also to the dimeric and monomeric forms of tmTNF-α, thus preventing the formation of the bioactive trimeric tmTNF-α (Scallon et al., unpublished data). In contrast, the p75-Fc construct binds only to the trimeric tmTNF-α. Moreover, it has been demonstrated that the p75-Fc construct plays a “ligand-passing” role by modulating the rate of TNF-α association with p55 TNFRI, thereby increasing the local concentration of TNF-α at the cell surface through rapid ligand association and dissociation [42]. A recent study by Frishman et al. [43] supported such a role for the p75-Fc receptor construct or native p75 receptor. In their study, both native soluble p75 and the p75-Fc construct increased TNF-α–induced IL-8 production in whole blood. Moreover, this increased IL-8 production was inhibited by native p55 TNFRI, further demonstrating the “ligand-passing” effect of the p75-Fc construct. Along this line, our findings show that anti–TNF-α MAB suppressed IL-8 production, whereas etanercept did not significantly affect the IL-8 level. Therefore, the modest increase in the incidence and cumulative swelling scores seen in the etanercept-treated group, compared with those in the saline-treated group, could result from a direct impact on TNF-α action at the local infection site or from an indirect effect, activation of other downstream inflammatory mediators via “ligand passing.”

Despite the immune response to anti–TNF-α MAB that was seen (figure 6), the reduction in abscess area by anti–TNF-α MAB treatment was still statistically significant on day 8 (figure 4), which suggests that the marked effect on abscess formation detected as early as day 3 in the animals treated with anti–TNF-α MAB in this acute infection model was maintained. Moreover, the establishment of an early suppression of abscess formation by anti–TNF-α MAB could explain the beneficial effect observed in the present study.
In summary, our findings demonstrate that the potent and specific neutralization of TNF-α by anti–TNF-α MAb delayed the onset and reduced the incidence and severity of S. aureus–induced abscess formation in cynomolgus monkeys and provide initial evidence that anti–TNF-α MAb therapy could benefit the host in defense against certain types of bacterial infections.

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