

The Combination of a Tumor Necrosis Factor Inhibitor and Antibiotic Alleviates Staphylococcal Arthritis and Sepsis in Mice

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(See the editorial commentary by Chow, on pages 332–4)

Background. Despite advances in medical practices, in recent decades permanent reductions in joint function have not been achieved, and the high mortality rate of patients with staphylococcal septic arthritis has not substantially improved.

Methods. We evaluated the effects of a combined tumor necrosis factor (TNF) inhibitor and antibiotic therapy on the course of *Staphylococcus aureus* arthritis and sepsis in mice.

Results. Treatment with the combination of a TNF inhibitor and an antibiotic resulted in a quicker relief of clinical arthritis in mice with septic arthritis, compared with an antibiotic monotherapy. Both histopathologically verified synovitis and the extent of joint destruction were reduced by this combined treatment. Importantly, anti-TNF treatment significantly improved the survival rate of mice with *S. aureus* sepsis and staphylococcal enterotoxin shock syndrome; this effect might be the result of a partial restoration of the hemostatic balance between coagulation and fibrinolysis. Finally, we demonstrated that anti-TNF treatment downregulates high-mobility group protein B1 in staphylococcal enterotoxin shock syndrome.

Conclusions. Thus, simultaneous systemic TNF inhibition and antibiotic therapy has beneficial effects on the outcome of *S. aureus* arthritis and sepsis in a mouse model, suggesting that the combination of a TNF inhibitor and antibiotics represents a novel therapeutic strategy for the treatment of staphylococcal infections.

Staphylococcus aureus septic arthritis remains a serious clinical problem that is associated with both chronic rheumatoid arthritis and immunodeficiency [1]. Despite advances in the use of antibiotics, permanent reductions in joint function due to joint deformation and deleterious contractures occur in ~40% of patients with septic arthritis [2, 3]. In addition, the high mortality rate of

5%–20% that is associated with this disease has not changed substantially in recent decades [4].

Postinfectious inflammatory sequelae are known to prolong joint destruction despite antibiotic treatment [5]. Tarkowski et al [6] demonstrated that the addition of corticosteroids to antibiotic treatments ameliorated the course of *S. aureus* septic arthritis in mice. Additionally, a combination of nonsteroidal anti-inflammatory drugs and antibiotics was shown to relieve *S. aureus*-induced arthritis in rabbits [7]. Tumor necrosis factor (TNF)- α , a central mediator of inflammation and immune regulation, has a detrimental effect in cases of systemic *S. aureus* infection. TNF/lymphotoxin- α double knockout mice are more resistant to septic arthritis than are the wild-type mice, although they exhibit increased mortality in response to *S. aureus* infection [8]. Protective effects against bone erosion by anti-TNF therapy in cases of rheumatoid

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arthritis indicate that this process of joint destruction is likely to be dependent on TNF- α [9]. Intriguingly, persistently high TNF- α levels in affected joints after appropriate antibiotic treatments were correlated with local complications of septic arthritis [10]. Therefore, we hypothesize that anti-TNF therapy in combination with antibiotics minimizes post-infectious sequelae and consequently reduces joint destruction in staphylococcal septic arthritis.

The primary aim of our study was to test the effects of a TNF inhibitor in combination with antibiotic therapy in the management of staphylococcal arthritis. Interestingly, the combination therapy reduced not only the severity of clinical arthritis but also the associated mortality rate. Thus, we further demonstrated that anti-TNF treatment improved the survival rate of mice with *S. aureus* sepsis and staphylococcal enterotoxin shock syndrome.

MATERIALS AND METHODS

Mice

Female NMRI and BALB/c mice at 6–8 weeks of age were purchased from Charles River Laboratories. They were housed under standard laboratory conditions. All animal experiments performed were approved by the animal ethics committee of Gothenburg University.

Bacterial Strains and Reagents

The *S. aureus* strain LS-1 [11] was prepared as described previously [12]. Human plasminogen and the plasmin-specific substrate S-2251 were purchased from Haemochrom Diagnostica. Toxin shock syndrome toxin-1 (TSST-1) from *S. aureus*, staphylococcal enterotoxin A (SEA), and *Escherichia coli* O55:B5 lipopolysaccharide (LPS) were purchased from Sigma Chemicals.

Experimental Protocols for Staphylococcal Septic Arthritis and Sepsis

Six separate *in vivo* experiments were performed for the staphylococcal septic arthritis studies. In all experiments, mice were inoculated intravenously into the tail vein with 0.2 mL of a staphylococci suspension.

In the first 5 experiments, we assessed the effect of combination therapy with a TNF inhibitor and antibiotics on staphylococcal arthritis ($n = 5$ –10 mice/group for each experiment). All mice were inoculated with an arthritogenic dose of 1.3 – 1.8×10^7 *S. aureus* strain LS-1 per mouse. During the course of the experiments, the mice were regularly weighed and examined for arthritis by observers blinded to the treatment groups (T. J. and Y. F.) until the mice were sacrificed. After sacrificing the mice at day 14, the kidneys were obtained for the assessment of bacterial persistence, serum samples were analyzed to assess cytokine levels, and the paws were microscopically evaluated for the expression of synovitis and destruction of cartilage and bone.

One experiment was performed to study the effect of combination therapy on staphylococcal sepsis ($n = 8$ mice/group).

Mice were inoculated with a septic dose of 6.2×10^7 *S. aureus* strain LS-1 per mouse. When a mouse was judged too ill to survive until the next time point, it was killed by cervical dislocation and considered dead due to sepsis.

Treatment With an Antibiotic and Anti-TNF Compound

Cloxacillin (Cloxacillinatr) dissolved in sterile phosphate buffered saline (PBS) was used for the antimicrobial treatments. The mice were injected with 0.2 mL of the solution (0.5 mg/g of body weight) intraperitoneally every 12 hours, starting at day 3 after inoculation with bacteria and continuing until the animals were killed. For the anti-TNF treatment, Enbrel (also known as etanercept; Wyeth Europa) was used. It has been shown that etanercept efficiently inhibits the activity of murine TNF- α [13, 14]. Etanercept was diluted in 0.2 mL of sterile PBS and injected subcutaneously every 48 hours at a dose of 5 μ g/g of body weight starting on day 3 after inoculation of bacteria and continuing until the animals were killed.

Clinical Evaluation of Arthritis

All 4 limbs of each mouse were visually inspected by observers who were blinded to the treatment groups. Arthritis was defined as erythema and/or swelling of the joints. To evaluate the severity of arthritis, a clinical scoring system of 0–3 was used as described previously [15] for each limb.

Experimental Protocols for Staphylococcal Enterotoxin-Induced Shock

Five separate experiments were performed to study staphylococcal enterotoxin-induced shock [16]. In all of the experiments, BALB/c mice were challenged with an intraperitoneal injection of 10 μ g of TSST-1 or SEA, followed 4 hours later by an intraperitoneal injection of 170 μ g of LPS. The numbers of deaths were recorded at frequent intervals in the first 4 experiments.

Etanercept (10 μ g/g of body weight) in 100 μ L of PBS was injected subcutaneously into mice either 10 minutes before the TSST-1 or SEA challenge (early treatment) or 6 hours after the SEA challenge (late treatment). Animals receiving the same volume of PBS served as controls.

In a fifth experiment, etanercept (10 μ g/g of body weight) in 100 μ L of PBS was injected subcutaneously into mice 10 minutes prior to SEA challenge. Mice were sacrificed either 7 or 24 hours after the SEA challenge. The blood, kidneys, lungs, livers, and distal ileums of these mice were collected.

Histopathologic Examination

Following staphylococcal enterotoxin-induced shock, the lungs, livers, kidneys, and distal ileums of the mice were processed, embedded in paraffin, and sectioned at 3–4- μ m intervals. Hematoxylin and eosin staining was performed to identify the tissues and detect alterations in cellular architecture. The tissues were examined by a pathology specialist (W. Z. W.) who was blinded to the treatment groups. Pathological changes in

liver tissues were assessed for congestion (grade, 0–3), lobular degeneration area (0%–100%), focal hepatocellular necrosis (yes/no), and Kuffer cell proliferation (yes/no). The lung tissues were assessed for congestion (grade, 0–3), alveolar edema or exudation (grade, 0–3), and fibrosis or sclerosis (grade, 0–3). The kidney tissues were assessed for congestion (grade, 0–3) and tubular degeneration (grade, 0–3). The intestinal tissues were assessed for mucosal congestion (yes/no), mucosal necrosis (yes/no), submucosal edema (yes/no), and muscle wall congestion (yes/no).

Histopathologic examinations of the joints were performed after routine fixation, decalcification, and paraffin embedding. Tissue sections were stained with hematoxylin and eosin, and the joints were studied microscopically to assess synovial hypertrophy and cartilage and/or bone destruction as described previously [15].

Bacteriologic Examination

The kidneys of the mice were removed, homogenized, diluted serially in PBS, and transferred to agar plates containing 5% (v/v) horse blood. Bacteria were grown for 24 hours and quantified as colony-forming units (CFUs).

Hematologic Analysis and Plasma Isolation Procedures

Blood samples were collected from mice into EDTA-containing tubes. Platelet and erythrocyte counts were analyzed. The collected blood samples were centrifuged at 800 g for 20 minutes, aliquoted, and stored in a -70°C freezer until further use.

Plasmin Activity Assay

Plasmin activity was determined by hydrolysis of the specific plasmin substrate S-2251 (H-D-Val-Leu-Lys-pNA.2HCL) as described previously [17].

Determination of Plasma Fibrinogen, PAI-1, and HMGB-1 Levels

The levels of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) in the plasma samples were measured using a mouse fibrinogen immunoperoxidase assay and a mouse PAI-1 total antigen assay (Innovative Research), respectively.

The levels of HMGB-1 in plasma were determined using a sandwich enzyme-linked immunosorbent assay kit (Shino-Test Corporation).

Statistical Analysis

The statistical significance of our data was assessed using the Mann–Whitney U test, the χ^2 test, and the log-rank survival test. The results are reported as the median and interquartile range or the mean \pm the standard error of the mean (SEM).

RESULTS

Effects of TNF Inhibitor and Antibiotic Treatments on Staphylococcal Arthritis

To study the effect of combination therapy on *S. aureus* arthritis, mice were inoculated with an arthritogenic dose of $1.3\text{--}1.8 \times 10^7$

S. aureus strain LS-1 per mouse (Figure 1A and 1B). On day 3 after inoculation with bacteria, $\sim 80\%$ of the mice had developed symptoms of arthritis. On day 7, the arthritis index of the animals receiving PBS or TNF inhibitor alone continued to increase. The severity of arthritis slightly increased in the group treated with the antibiotic alone. By contrast, the mice treated with the combination therapy displayed clear decreases in both the arthritis index and the frequency of arthritis. These differences became even more evident during the late stage of the disease. There was a tendency at day 10 that mice treated with TNF inhibitor and antibiotics showed a reduced arthritis frequency (68% vs 89%; $P = .07$) and a reduced arthritis index (1.24 vs 1.91; $P = .06$), compared with mice that received the antibiotic alone. A significantly lower arthritis index was observed at day 14 in mice that received combination therapy than in mice treated with the antibiotic alone (0.9 vs 1.67; $P = .015$). Importantly, these results were in agreement with the histopathologic findings (Figure 1C and 1D). Additionally, the destruction of bone and cartilage was also significantly reduced in the combination therapy group, compared with that in the mice receiving antibiotic alone.

Surprisingly, the overall mortality was not affected by the antibiotic treatment by the end of the experiments (Figure 2A), whereas the cumulative mortality was significantly lower in the combination therapy group than in the antibiotic group (14% vs 55%; $P < .01$).

Weight loss percentage is a useful parameter for monitoring the severity of staphylococcal septic arthritis. Mice that received either PBS or anti-TNF therapy lost up to 20% of their body weight during the experimental period (Figure 2B). By contrast, mice that received either combination therapy or the antibiotic alone started gaining weight after treatment initiation and reached $\sim 95\%$ of their original body weight by the end of the experiments. No difference was identified between the antibiotic group and the combination therapy group.

Sixty-three percent of the kidneys from the PBS-treated group and 100% of the kidneys of mice that received anti-TNF therapy alone harbored staphylococci (Figure 2C; $P < .01$); by contrast, bacteria were not found in the kidneys of the other 2 treatment groups (Figure 2D). More bacteria were present in the kidneys of the mice that received TNF inhibitor than in those that received PBS ($P = .07$).

Effects of Antibiotic and Anti-TNF Treatments on Staphylococcal Sepsis

To further study the effect of combination therapy on *S. aureus* lethal sepsis, antibiotics and etanercept were given to mice inoculated with a septic dose of 6.2×10^7 CFU of *S. aureus* strain LS-1 per mouse. All of the animals treated with the antibiotic alone died within 7 days after infection, whereas $>60\%$ of mice

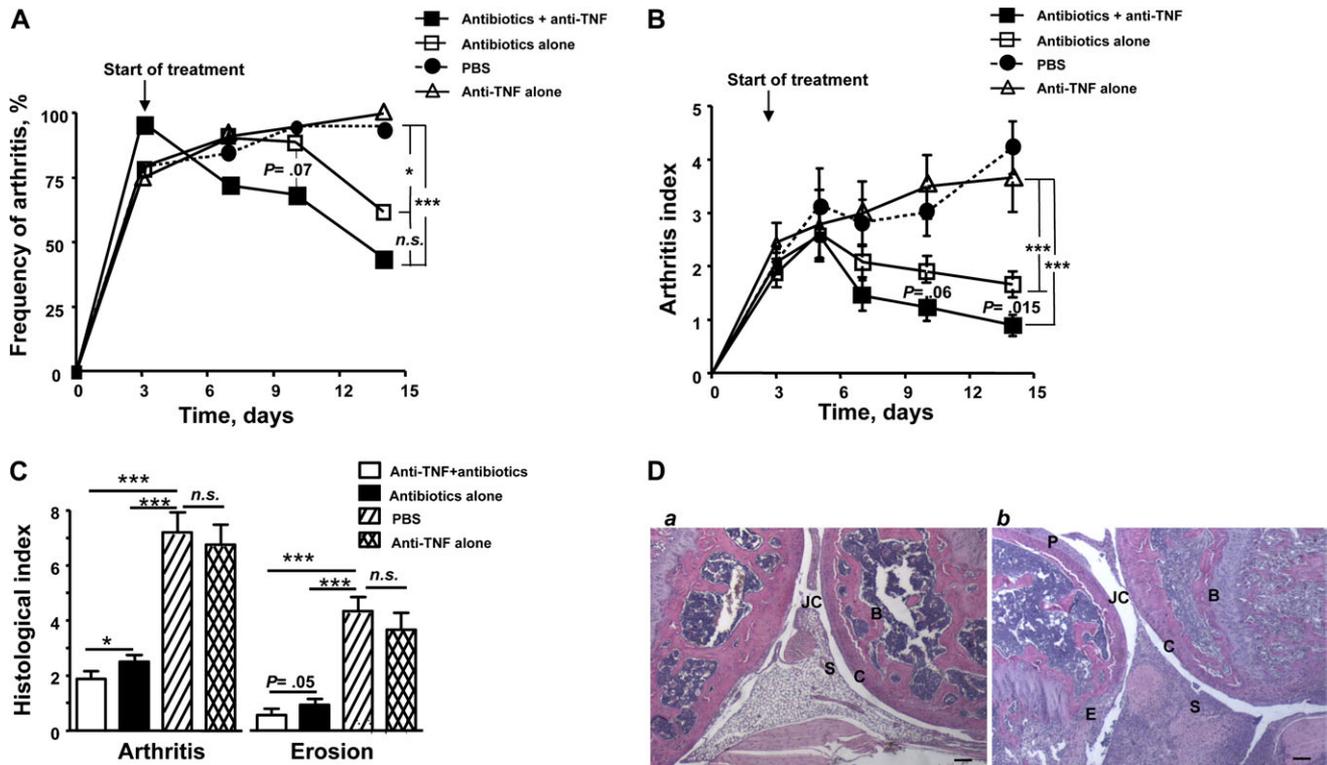


Figure 1. The effect of anti-tumor necrosis factor (TNF) and antibiotic treatments on staphylococcal arthritis. NMRI mice inoculated with *Staphylococcus aureus* LS-1 ($1.3\text{--}1.8 \times 10^7$ colony-forming units [CFU]/mouse) were treated with cloxacillin and etanercept (anti-TNF) ($n = 24\text{--}31$ mice/group) starting on day 3 after inoculation with bacteria and continuing until the animals were killed on day 14. The frequency (A) and severity (B) of arthritis in the mice were observed for 14 days after infection. Y values represent measurements from surviving mice only for the respective days. The values from 5 experiments were pooled. The Mann-Whitney *U* test was used to evaluate the statistical significance of the results. Data were presented as mean values \pm standard error of the mean (SEM). C, Histological evaluation of the joints from all 4 limbs of NMRI mice that were sacrificed 14 days after infection. Comparisons were made using the 1-tailed Mann-Whitney *U* test. Data were presented as mean values \pm SEM. D, A micrograph of histologically intact knee joints from a NMRI mouse that was given antibiotics and anti-TNF treatments 14 days after inoculation with 1.3×10^7 CFU of *S. aureus* LS-1 (a) and a micrograph of a heavily inflamed knee joint with severe bone and cartilage destruction from a NMRI mouse with septic arthritis treated with PBS (b). Hematoxylin and eosin was used to stain the following tissues: B, bone; C, cartilage; E, erosion of bone and cartilage; JC, joint cavity; P, pannus formation; S, synovial tissue. The bar represents 100 μm . * $P < .05$; *** $P < .001$. ns, not significant; PBS, phosphate buffered saline.

in the combination therapy group survived and recovered completely (Figure 3; $P = .017$).

Anti-TNF Therapy Prevents Staphylococcal Enterotoxin-triggered Death

In addition to septic arthritis and sepsis, *S. aureus* are also able to produce severe toxic shock syndrome through toxin production. To investigate whether anti-TNF protects enterotoxin shock syndrome, etanercept was given to BALB/c mice challenged with staphylococcal enterotoxins (TSST-1 and SEA). Early treatment of anti-TNF significantly prolonged median survival in TSST-1-induced shock, compared with the PBS group (Figure 4A; $P < .001$). Figure 4B shows that both early and late treatment with the TNF inhibitor led to significantly improved median survival rates, compared with the control group, in SEA-induced shock. Injection of mice with SEA/LPS resulted in 100% mortality within 40 hours. By contrast, 9%–20% of

mice that received etanercept were still alive 86 hours after the SEA challenge.

Histopathological Changes in Internal Organs Following Anti-TNF Treatment in SEA Shock Syndrome

Internal organs, including the livers, lungs, kidneys, and distal ileums, were collected for histopathological staining 24 hours after the SEA challenge. Significant differences were identified in liver tissues with respect to the lobular degeneration area percentage and focal necrosis and in kidney tissues with respect to tubular degeneration between anti-TNF and PBS groups (Table 1). The lobular degeneration area in liver was increased by anti-TNF treatment (32% vs 78%; $P = .004$), whereas focal necrosis was protected ($P = .061$). In the kidneys, a significantly lower extent of tubular degeneration was found in the anti-TNF treatment group than in the PBS group ($P = .017$).

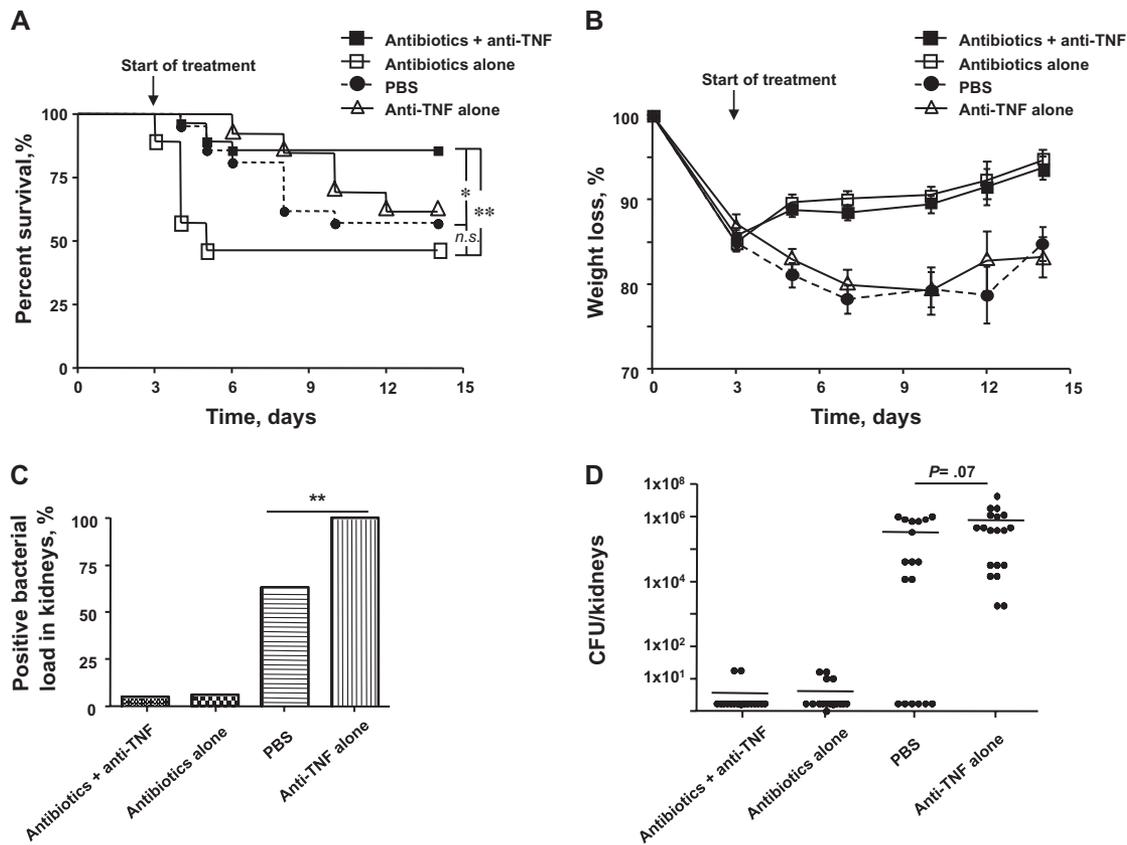


Figure 2. The effect of anti-tumor necrosis factor (TNF) and antibiotic treatments on the clinical course of staphylococcal arthritis. NMRI mice inoculated with *Staphylococcus aureus* LS-1 ($1.3\text{--}1.8 \times 10^7$ colony-forming units [CFU]/mouse) were treated with cloxacillin and etanercept (anti-TNF) ($n = 24\text{--}31$ mice/group) starting on day 3 after inoculation with bacteria and continuing until the animals were killed on day 14. Cumulative survival (A) and changes in percentage body weight (B) of the mice were observed for 14 days after infection. Y values represent measurements from surviving mice only for the respective days. The values from 5 different experiments were pooled. Statistical evaluations were performed using the Kaplan-Meier log-rank test and the Mann-Whitney *U* test. Data were presented as mean values \pm standard error of the mean. C, Positive bacterial load in kidneys 14 days after infection. D, Persistence of *S. aureus* in kidneys 14 days after infection. Values from 5 experiments were pooled. Statistical evaluations were performed using the Mann-Whitney *U* test. * = $P < .05$; ** = $P < .05$ by Fisher's exact test; *** = $P < .001$. ns, not significant; PBS, phosphate buffered saline.

Effects of Anti-TNF Treatment on Hemostatic Markers in SEA-induced Shock

Thrombopenia and low fibrinogen levels in the blood indicate disseminated intravascular coagulation (DIC) and poor prognosis of sepsis. Anti-TNF treatment significantly increased both the platelet counts (Figure 5A; $P < .01$) and the fibrinogen levels (Figure 5B; $P < .01$) in the blood of animals, suggesting that anti-TNF treatment prevents the development of DIC in SEA-induced shock.

Impaired fibrinolysis is a hallmark of pathogenesis in septic shock. Notably, the activity of plasmin was dramatically decreased to 6.9% of the activity level in healthy control animals 24 hours after the SEA challenge (Figure 5C; $P < .01$). Treatment with the TNF inhibitor partially restored the plasmin activity back to 12.5% ($P = .06$).

PAI-1, one of the major inhibitors of fibrinolysis, was increased 50-fold in mice 7 hours after the SEA challenge (Figure

5D; $P < .01$). Surprisingly, PAI-1 levels were even higher in mice that received anti-TNF therapy, compared with control animals, 24 hours after the SEA challenge ($P < .05$), which suggested that the restoration of plasmin activity by anti-TNF treatment might not have been mediated by the PAI-1 pathway.

Anti-TNF Treatment Reduces HMGB-1 Levels in SEA-induced Shock

HMGB-1 mediates lethality resulting from endotoxemia and experimental sepsis [18, 19]. The levels of HMGB-1 in the plasma of mice 24 hours after the SEA challenge are shown in Figure 6. Mice challenged with SEA had significantly higher HMGB-1 levels than did healthy controls (mean level \pm SEM, 107.5 ± 19.4 ng/mL vs 13.1 ± 3.4 ng/mL; $P < .05$). There was a clear reduction in HMGB-1 levels (mean level \pm SEM, 33.4 ± 3.5 ng/mL) in the plasma of mice that received anti-TNF therapy ($P < .05$).

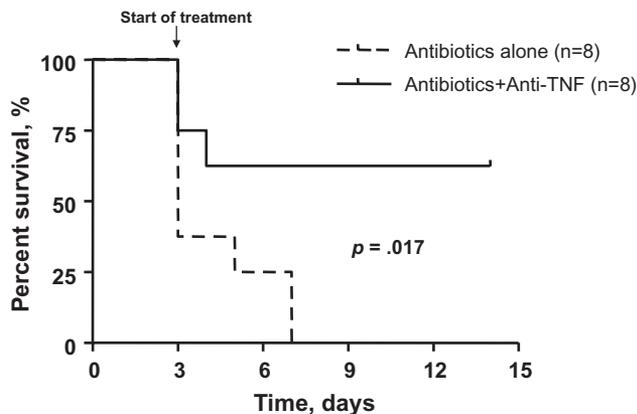


Figure 3. Effect of anti-tumor necrosis factor (TNF) and antibiotic treatments on staphylococcal sepsis. NMRI mice inoculated with a septic dose of 6.2×10^7 colony-forming units of the *Staphylococcus aureus* strain LS-1 per mouse were treated with cloxacillin and etanercept (anti-TNF) ($n = 8$ mice/group) starting on day 3 after inoculation of bacteria and continuing until day 14. Statistical evaluations were performed using the Kaplan-Meier log-rank test.

DISCUSSION

In this study, we demonstrated that a combination therapy with an antibiotic and a TNF inhibitor significantly alleviates staphylococcal septic arthritis and sepsis, compared with an antibiotic monotherapy. Mice receiving the combination therapy exhibited significantly faster relief from the symptoms of clinical arthritis than did those that were given an antibiotic alone. Histopathologically verified synovitis and the extent of joint destruction were both reduced by this combined treatment.

Importantly, anti-TNF treatment improved the survival rate of mice with *S. aureus*-induced sepsis and staphylococcal enterotoxin-induced shock. The hemostatic imbalance between coagulation and fibrinolysis was partially restored by anti-TNF therapy. Finally, we demonstrated that anti-TNF treatment downregulates HMGB-1 expression in mice with staphylococcal enterotoxin shock syndrome.

There is a subtle balance between an effective immune response eliminating infecting organisms and an overactive immune response causing infection-related joint destruction. Reducing postinfectious inflammation by adding a non-steroidal anti-inflammatory drug [7] or corticosteroid [6] to a standard antibiotic regimen reduces the levels of cartilage damage that results from *S. aureus*-induced arthritis. TNF- α drives the cytokine cascade and induces the release of matrix metalloproteinase (MMP) enzymes [20] from a wide range of cells in the synovial tissue. The neutralization of TNF- α reduces levels of MMP1 and MMP9 in patients with rheumatoid arthritis [21] and downregulates many other pro-inflammatory cytokines [22]. Importantly, TNF- α levels in synovial fluid from affected joints in patients with septic arthritis are persistently high even 7 days after the initiation of antibiotic treatment [10]; this finding may explain the insufficiency of antibiotic monotherapy in treating septic arthritis [5] and the clear benefit of the combination therapy in preventing joint inflammation and bone damage that was observed in the present study. It is important to be aware of the significantly higher mortality rate in the antibiotic group, compared with that in the combination therapy group. Although this difference might have resulted in a sampling bias when we compared the clinical and pathological arthritis

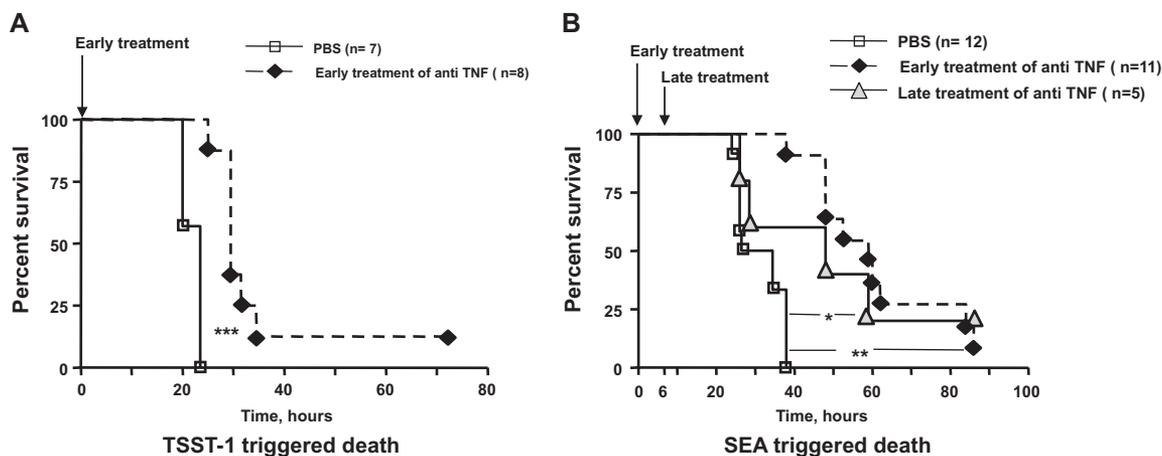


Figure 4. Prolonged survival by anti-tumor necrosis factor (TNF) treatment in a murine model of staphylococcal enterotoxin-induced death. BALB/c mice were challenged intraperitoneally with toxin shock syndrome toxin-1 *Escherichia coli* O55:B5 lipopolysaccharide (TSST-1-LPS) (A) and staphylococcal enterotoxin A (SEA)-LPS (B). Etanercept (10 μ g/g of body weight) in 100 μ L of phosphate-buffered saline (PBS) was injected subcutaneously to mice 10 minutes before enterotoxin challenge (early treatment, \blacklozenge) or 6 hours after enterotoxin challenge (late treatment, \blacktriangle). Mice that were administered the same volume of PBS served as controls (\square). Data are expressed as survival of mice during the first 90 hours after enterotoxin challenge. The data in B were pooled from independent experiments. * = $P < .05$; ** $P < .01$; *** $P < .01$ by the Kaplan-Meier log-rank test.

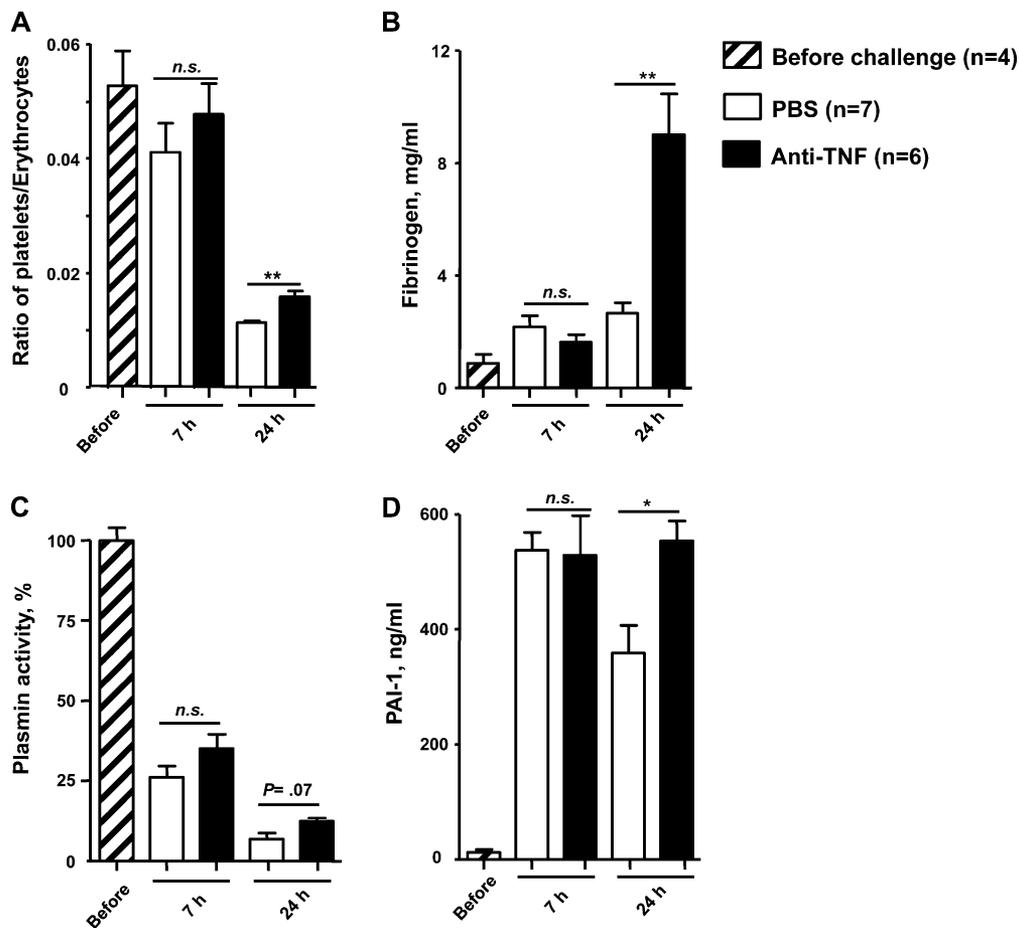


Figure 5. The effects of anti-tumor necrosis factor (TNF) on hemostatic markers in staphylococcal enterotoxin A (SEA)-induced shock. BALB/c mice were challenged intraperitoneally with SEA-*Escherichia coli* O55:B5 lipopolysaccharide (LPS) ($n = 4-7$ mice/group). Etanercept (10 $\mu\text{g/g}$ of body weight) in 100 μL of phosphate-buffered saline (PBS) was injected subcutaneously into mice 10 minutes before the SEA challenge. Mice injected with the same volume of PBS served as controls. Blood was collected at both 7 and 24 hours after the SEA challenge. The ratio of platelets/erythrocytes (A), fibrinogen levels (B), plasmin activity levels (C), and plasminogen activator inhibitor-1 levels (D) in blood from mice with SEA-induced shock at 7 and 24 hours after the SEA challenge. Statistical evaluations were performed using the Mann-Whitney U test. Data were presented as mean values \pm standard error of the mean. * = $P < .05$; ** = $P < .01$. ns, not significant.

scores between these 2 groups, the mice in the antibiotic group that were omitted due to death would likely have had the most-severe infections. Therefore, the protective effect of anti-TNF therapy against joint destruction might have appeared even more pronounced if this sampling bias had not occurred.

Anti-TNF therapy has shown limited efficacy against septic shock in clinical trials [23], possibly because TNF is an early mediator of sepsis pathogenesis. However, we observed improved survival rates in cases of staphylococcal sepsis when animals were given a combination of anti-TNF therapy and an antibiotic. There is a possible explanation for this inconsistency, however. We observed that, in animals that received only the antibiotic, the instances of death were concentrated within a short period (1-3 days) soon after antibiotic administration. By contrast, the deaths of mice that

received PBS occurred in a more dispersed fashion over the entire course of the disease. Thus, antibiotic administration during a short time window after treatment initiation tended to accelerate the course of septic shock. It is known that exposure of staphylococci to β -lactam antibiotics greatly enhances the release of bacterial cell wall fragments that have strong proinflammatory properties, such as peptidoglycan and lipoteichoic acid [24, 25], resulting in the induction of higher TNF levels from monocytes [26] and increased endothelial chemokine secretion and adhesiveness of granulocytes [27]. This may not have significant clinical relevance in cases of minor infection; however, in animals that are already in a state of septic shock, the higher cytokine production levels might exacerbate the imbalance and lead to unexpected death. Indeed, the observed protective effect of the TNF inhibitor strongly suggests that TNF is the mediator that is most responsible for the rapid death of

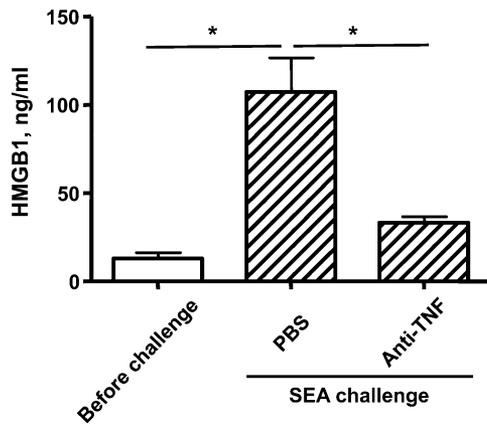


Figure 6. Anti-tumor necrosis factor (TNF) treatment downregulates HMGB-1 levels in mice with staphylococcal enterotoxin A (SEA)-induced shock. BALB/c mice were challenged intraperitoneally with SEA-*Escherichia coli* O55:B5 lipopolysaccharide (LPS) ($n = 4-5$ mice/group). Etanercept (10 $\mu\text{g/g}$ of body weight) in 100 μL of phosphate-buffered saline (PBS) was injected subcutaneously to mice 10 minutes before the SEA challenge. Mice injected with the same volume of PBS served as controls. Blood was collected 24 hours after the SEA challenge. The levels of HMGB-1 in the blood were analyzed by enzyme-linked immunosorbent assay. Statistical evaluations were performed using the Mann-Whitney U test. Data were presented as mean values \pm SEM. * = $P < .05$.

septic animals receiving antibiotics. However, the clinical significance of antibiotic-induced staphylococcal cell wall fragment release, specifically the association of such release with pathogens, mortality, and alterations in physiological parameters in humans, must be further elucidated.

Components of staphylococcal cell walls and staphylococcal enterotoxins share one common feature: They are both strong inducers of proinflammatory cytokines (eg, TNF- α). Exposure to staphylococcal enterotoxins leads to toxic shock syndrome, which is an acute, multisystem illness, often resulting in multi-organ failure [28]. Inhibition of fibrinolysis is a key element of the pathogenesis of fibrin deposition in septic shock. It has been shown that early inhibition of activated fibrinolysis and high levels of D-dimer correlate with the fatal outcomes of some infectious diseases [29, 30]. In the present study, significantly

decreased plasmin activities and elevated PAI-1 levels in the blood indicate an impaired fibrinolysis in mice with toxic shock syndrome. Intriguingly, the TNF inhibitor used in this study partially restored plasmin activity. PAI-1 levels, however, were upregulated by anti-TNF treatment, suggesting that pathways that are not affected by the blockade of PAI-1 release are involved in the restoration of fibrinolysis.

HMGB-1 is implicated as a late mediator of endotoxin lethality that exhibits significantly delayed kinetics relative to TNF [18]. Neutralizing HMGB-1-specific antibodies prevents the lethality resulting from established sepsis in animals [19]. Both LPS and TNF- α induce the secretion of HMGB-1 [31]. Staphylococcal enterotoxin (eg, TSST-1) is also able to induce translocation and secretion of HMGB-1 requiring the activation of both T cells and monocytes [32]. Indeed, we found that anti-TNF reduced plasma concentrations of HMGB-1 to almost normal levels in mice with SEA shock syndrome, which suggested that TNF- α is the major mediator of HMGB-1 expression in this animal model. However, it remains unclear whether the observed effect in blocking HMGB-1 secretion by anti-TNF is mediated through inhibition of SEA-mediated signaling, LPS signaling, or perhaps both molecules.

Patients treated with TNF inhibitors are at an increased risk of developing certain infections [33-36]. It is recommended that the use of anti-TNF therapy during severe infections in clinical settings should be discontinued. In the present study, we did not observe any possible septic complications when the TNF inhibitor was used together with effective antibiotics. In fact, the combined treatment resulted in a significantly reduced mortality rate in models of both staphylococcal septic arthritis and sepsis, suggesting that the combination therapy is safe. However, it is important to consider the potential dangers associated with clinicians choosing inadequate antibiotics in combination with TNF inhibitors before receiving microbiological culture results. Although anti-TNF therapy alone significantly increased the levels of bacterial growth in kidneys, compared with the PBS-treated group, it did not affect the clinical outcomes of infection (ie, arthritis severity, weight development, or overall survival) when the anti-TNF

Table 1. Pathological Changes in the Kidneys and Livers of Mice With Staphylococcal Enterotoxin A (SEA)-Induced Shock After Anti-Tumor Necrosis Factor (TNF) Treatment

| Organ, pathological changes | PBS ($n = 5$) | TNF inhibitors ($n = 6$) | P |
|--|-----------------|----------------------------|------|
| Liver | | | |
| Area of lobular degeneration, mean % (SEM) | 32.0 (4.9) | 78.3 (4.0) | .004 |
| No. of mice with focal necrosis (no. of mice with no necrosis) | 3 (2) | 0 (6) | .061 |
| Kidney | | | |
| Extent of tubular degeneration, mean grade (SEM) | 2.1 (0.10) | 1.16 (0.17) | .017 |

NOTE. BALB/c mice were challenged intraperitoneally with SEA-*Escherichia coli* O55:B5 lipopolysaccharide. Etanercept (10 $\mu\text{g/g}$ of body weight) in 100 μL of phosphate-buffered saline (PBS) was injected subcutaneously to mice 10 minutes before the SEA challenge. Mice injected with the same volume of PBS served as controls. The organs were collected 24 hours after the SEA challenge. Pathological changes were evaluated in liver tissues with respect to the lobular degeneration area (0%-100%) and focal necrosis (yes/no) and in kidney tissues with respect to extent of tubular degeneration (grade, 0-3). SEM, standard error of the mean.

treatment was initiated 3 days after infection. However, it is important to consider that there might be a time-related deteriorative effect associated with anti-TNF treatment in cases of staphylococcal infection. Data described by Nakane et al [37] and our unpublished data have shown that pretreatment with a TNF inhibitor significantly worsens the outcomes of staphylococcal infections.

Our data demonstrate clear clinical benefits of antibiotic and TNF inhibitor combination therapies relating to both arthritis severity and bone destruction in addition to reductions in the mortality rate by a combination therapy in experimental *S. aureus*-induced septic arthritis. Our results suggest that *S. aureus* septic arthritis should be considered a new potential indication for the use of TNF inhibitors and that a combination of TNF inhibitors and antibiotics might represent a novel therapeutic approach to treat *S. aureus*-induced septic arthritis.

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