

CTLA4 Immunoglobulin but Not Anti-Tumor Necrosis Factor Therapy Promotes Staphylococcal Septic Arthritis in Mice

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Background. The development of biologics has greatly increased the quality of life and the life expectancy of many patients with rheumatoid arthritis. However, a large number of these patients have an increased risk of developing serious infections. The aim of this study was to examine differential effects of anti-tumor necrosis factor (TNF) treatment and CTLA4 immunoglobulin (Ig) treatment on both immunological response and host defense in a murine model of septic arthritis.

Methods. Abatacept (CTLA4-Ig), etanercept (anti-TNF), or phosphate-buffered saline were given to NMRI mice intravenously inoculated with *Staphylococcus aureus*. The clinical course of septic arthritis and histopathological and radiological changes of joints were compared among the groups.

Results. Mice receiving CTLA4-Ig treatment had more-severe septic arthritis, compared with controls and mice receiving anti-TNF treatment. Anti-TNF treatment led to more-severe weight loss and kidney abscesses, as well as a higher bacterial burden in the kidneys. Mice receiving CTLA4-Ig therapy had lower serum levels of interleukin 4, whereas mice receiving anti-TNF therapy had higher levels of TNF- α . Both iNOS and arginase-1 expression were reduced in peritoneal macrophages from mice receiving CTLA4-Ig, compared with expression in the anti-TNF group.

Conclusions. CTLA4-Ig therapy significantly increased the susceptibility to *S. aureus* septic arthritis in mice, whereas anti-TNF therapy deteriorated host bacterial clearance, resulting in more-severe weight loss and kidney abscesses.

Keywords. *Staphylococcus aureus*; septic arthritis; mouse; abatacept; etanercept.

Septic arthritis is still a major medical challenge because of its high prevalence, rapidly progressive disease character, and poor prognosis regarding joint functions. In developed countries, the yearly reported incidence of septic arthritis varies from 4 to 10 cases per 100 000 person-years in the normal population. The fatality

rate for septic arthritis is high (5%–15%), and permanent loss of joint functions develops in 25%–50% of patients. *Staphylococcus aureus* is the most common cause of bacterial arthritis. The risk factors for acquiring septic arthritis include increasing age, decreased immunocompetence, and preexisting joint diseases [1]. Patients with rheumatoid arthritis are at high risk for septic arthritis, owing to factors related to the disease itself and to the use of immunosuppressive drugs. A prospective study demonstrated that patients with rheumatoid arthritis have a 4-fold increased risk of septic arthritis [2], but this study was done before the biological agents became established in routine rheumatoid arthritis care.

During the past 2 decades, advances in the understanding of inflammation pathogenesis in autoimmune arthritis have led to the successful use of biologics that target specific molecules and cells of the immune response. Several biologic products that either inhibit the actions of

Received 14 January 2015; accepted 25 March 2015.

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The Journal of Infectious Diseases®

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DOI: 10.1093/infdis/jiv212

proinflammatory cytokines (tumor necrosis factor α [TNF- α] and interleukin 6 [IL-6] inhibitors), modulate the activation of T cells (CTLA4 immunoglobulin [Ig]) or deplete B cells (anti-CD20 therapy) are now available to treat patients with rheumatoid arthritis [3]. So far, anti-TNF therapy is the first choice of the biologics given to patients with rheumatoid arthritis with inadequate response to methotrexate. However, CTLA4-Ig treatment was recently shown to have an efficacy profile similar to that of anti-TNF therapy with regard to clinical, functional, and radiographic outcomes [4]. Several lines of evidence suggested a higher risk of serious infections in patients with rheumatoid arthritis treated with anti-TNF- α therapy, compared with patients treated with CTLA4-Ig [4–6]. However, the susceptibility to a particular infection, such as septic arthritis, may differ from the other infections, and there was no study designed to compare the risk for septic arthritis between anti-TNF and CTLA4-Ig treatments.

It is known that both TNF- α and CD4⁺ T cells play vital roles in pathogenesis of *S. aureus* septic arthritis. TNF/lymphotoxin- α double-knockout mice are more resistant to septic arthritis than wild-type mice [7]. CD4⁺ T cells are known to be pathogenic during *S. aureus* arthritis in mice, and pretreatment with anti-CD4 antibodies attenuated the severity of *S. aureus* septic arthritis in mice [8, 9]. CTLA4-Ig treatment interrupts the CD28/B7 costimulatory pathway that is essential for the development and homeostasis of regulatory T cells and accelerates some immune-mediated diseases [10–12]. Thus, we hypothesized that both anti-TNF and CTLA4-Ig treatment may influence the induction and maintenance of septic arthritis.

In this study, we sought to examine differential effects of anti-TNF and CTLA4-Ig pretreatment on both immunological responses and host defense in a well-established mouse septic arthritis model that closely resembles human *S. aureus* septic arthritis [13]. Our data demonstrate that CTLA4-Ig therapy but not anti-TNF treatment greatly aggravated staphylococcal septic arthritis in mice. In contrast, anti-TNF treatment led to more weight loss and impaired bacterial clearance in kidneys.

MATERIALS AND METHODS

Mice

Female NMRI mice aged 6–8 weeks were purchased from Charles River Laboratories (Sulzfeld, Germany). They were bred and housed in the animal facility of the Department of Rheumatology and Inflammation Research, University of Gothenburg. Mice were kept under standard conditions of temperature and light and were fed laboratory chow and water ad libitum. The ethics committee of animal research of the University of Gothenburg approved the study.

Experimental Protocols for Staphylococcal Septic Arthritis

Staphylococcus aureus Newman strains were prepared as previously described [14]. We used a well-established mouse model

of septic arthritis to closely resemble the human infectious arthritis that spreads hematogenously [13]. To evaluate the effect of a TNF inhibitor and CTLA4-Ig therapy on staphylococcal arthritis, outbred NMRI mice, rather than inbred mice, were used to more accurately mimic what one would find in humans. Mice were intravenously inoculated with 0.2 mL of staphylococcal suspension of Newman strain into the tail vein and were euthanized on day 10 after inoculation.

Three experiments were performed to study the effect of a TNF inhibitor and CTLA4-Ig therapy on staphylococcal arthritis. All mice (5–11/group) were inoculated with an arthritogenic dose of 1.7×10^6 *S. aureus*. The mice were regularly weighed and examined for arthritis. After mice were euthanized, on day 10, the kidneys, serum, and the paws were collected. Because all experiments had similar outcomes, the results were pooled.

To study the effect of anti-TNF and CTLA4-Ig on bacterial load locally in the joints, mice (10/group) inoculated with *S. aureus* Newman strain (1.7×10^6 colony-forming units [CFU]/mouse) were regularly examined for arthritis. The joints (wrists, ankles, and knees) that are most often affected by *S. aureus* were collected and homogenized for CFU counts on day 5, when the clinical difference in arthritis severity became evident.

Treatment with TNF- α Inhibitor and CTLA4-Ig

Etanercept (Enbrel; Wyeth Europa) was used for the anti-TNF treatment because it fully inhibits the biologic function of murine TNF [15]. Abatacept (Orencia; Bristol-Myers Squibb), a fusion protein of CTLA4-Ig, was used to modulate the costimulation of T cells in mice [16]. Etanercept (5 μ g/g of body weight) or abatacept (0.25 mg/g of body weight) in 0.1 mL of phosphate-buffered saline (PBS) were given subcutaneously twice weekly, starting 1 week before intravenous inoculation of *S. aureus* and continuing until mice were euthanized, on day 10.

Clinical Evaluation of Arthritis

Observers (T. J. and A. A.) blinded to the treatment groups visually inspected all 4 limbs of each mouse. Arthritis was defined as erythema and/or swelling of the joints. A clinical scoring system ranging from 0 to 3 was used for each paw (0, no inflammation; 1, mild visible swelling and/or erythema; 2, moderate swelling and/or erythema; and 3, marked swelling and/or erythema). The arthritis severity index was constructed by adding the scores from all 4 limbs for each animal as described before [15, 17]. Arthritis that involved ≥ 2 joints simultaneously was defined as polyarthritis.

Bacteriologic Examination

The kidneys were aseptically removed and blindly assessed by one investigator (T. J.) for abscesses. A scoring system ranging from 0 to 3 was used (0, healthy kidneys; 1, 1–2 small abscesses on kidneys without structure changes; 2, >2 abscesses but <75% of kidney tissue involved; and 3, large amounts of abscesses with >75% of kidney tissue involved). The kidneys or joints were

homogenized, diluted serially in PBS, and cultured for 24 hours on agar plates containing 5% horse blood. Bacteria were quantified as CFU.

Microcomputed Tomography (Micro-CT)

The joints were fixed in 4% formaldehyde for 3 days and then transferred to PBS for 24 hours. All 4 limbs were scanned and reconstructed into a 3-dimensional structure with Skyscan1176 micro-CT (Bruker, Antwerp, Belgium) with a voxel size of 35 μm . The scanning was done at 55 kV/455 mA, with a 0.2-mm aluminum filter. Exposure time was 47 ms. The X-ray projections were obtained at 0.7° intervals with a scanning angular rotation of 180°. The 3-dimensional images were reconstructed using NRECON software (version 1.5.1; Bruker) and analyzed with CT Analyzer (version 1.7; Bruker). The 3-dimensional structures of each joint were blindly assessed by 2 observers (T. J. and A. A.), using a scoring system from 0 to 3 (0, healthy joint; 1, mild bone destruction; 2, moderate bone destruction; and 3, marked bone destruction).

Histopathological Examination of Joints

The joints were decalcified, embedded in paraffin, and sectioned with a microtome. Tissue sections were stained with hematoxylin and eosin. All slides were coded and assessed in a blinded manner by 3 observers (T. J., M. M., and A. A.) with regard to the degree of synovitis and cartilage-bone destruction. The extent of synovitis and cartilage-bone destruction was judged as previously described [15, 17].

Characterization of Intraperitoneal Leukocytes

Mice treated with etanercept ($n = 12$), abatacept ($n = 12$), or PBS ($n = 13$) as described above were intraperitoneally injected with heat-killed *S. aureus* strain Newman (1×10^9 dead bacteria) in 200 μL of PBS. One day or 3 days later, they were euthanized, and peritoneal leukocytes were collected using peritoneal lavage with 10 mL of ice-cold PBS. Peritoneal lavage fluid was collected for cytokine analysis, and peritoneal cells were quantified and characterized.

Macrophages were stained with FITC-conjugated rat antimouse CD11b antibody (BD) and PE-Cy7-conjugated rat antimouse F4/80 antibody (eBioscience). Unspecific binding was blocked using Fc-block (BioLegend). Neutrophils were identified using APC-conjugated rat antimouse Ly6G antibody (BD). Peritoneal cells were fixed using 2% paraformaldehyde and were permeabilized with a 1:1 mixture of ice-cold acetone and methanol, and iNOS was stained using rabbit antimouse iNOS antibody (Abcam), detected by secondary Alexa Fluor 647-conjugated goat antirabbit antibody (Life). Simultaneously, arginase-1 was stained using a fluorescein-conjugated sheep antimouse arginase-1 antibody (R&D Systems, Abingdon, United Kingdom). The cells were analyzed on a BD Accuri flow cytometer. Macrophages were gated on the basis of F4/80-expression, and iNOS and arginase-1

expression were analyzed in terms of median fluorescence intensities. Isotype controls showed low unspecific binding.

Measurement of Cytokine Levels

The cytokine levels in serum were determined using the Cytometric Bead Array Mouse Th1/Th2/Th17 Cytokine Kit (BD Biosciences) and were analyzed using the FACS Canto2 flow cytometer. The levels of cytokines in peritoneal fluids and cell culture supernatants and the level of receptor activator of nuclear factor $\kappa\text{-B}$ ligand (RANKL) in serum were quantified using DuoSet enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems).

Statistical Analysis

Statistical significance was assessed using the Mann-Whitney U test and the χ^2 test. The results are reported as the mean values \pm the standard errors of the mean. A P value of $<.05$ was considered statistically significant.

RESULTS

CTLA4-Ig Treatment Significantly Increases the Severity and Frequency of Septic Arthritis

CTLA4-Ig-treated mice but not anti-TNF-treated mice developed significantly more-severe clinical arthritis, compared with the control group. The difference was clear by day 2 and increased over time until stabilizing at the end of the experiment (Figure 1A).

The frequency of arthritis was also higher in the CTLA4-Ig-treated mice, compared with the PBS-treated controls. On day 5, 71% of CTLA4-Ig-treated mice had developed arthritis, compared with 46% of the PBS-treated controls, and on day 10 the difference became statistically significant ($P = .03$; Figure 1B). In addition, the incidence of polyarthritis among CTLA4-Ig-treated mice was greater than that among PBS-treated controls, and the difference was statistically significant on days 7 ($P = .006$) and 10 ($P = .04$). No difference was found between anti-TNF-treated mice and the PBS-treated controls with regard to polyarthritis (data not shown).

Although treatment with CTLA4-Ig increased both arthritis severity and frequency, it did not affect weight loss, compared with PBS treatment (Figure 1C). In contrast, anti-TNF-treated mice had lost a greater percentage of baseline weight on day 7 (decrease from day 0, 18%), compared with the PBS-treated controls (decrease from day 0, 12%; $P = .06$), and the difference became more apparent on day 10 ($P = .04$).

The 3-dimensional joint structures and microscopic signs of arthritis were semiquantitatively assessed by 2 observers who were blind to the treatment groups, using a scoring system from 0 to 3 (Figure 2). In line with the clinical arthritis data above, micro-CT revealed that CTLA4-Ig-treated mice had a tendency toward more bone erosion ($P = .06$; Figure 2A), and histopathological synovitis and joint destruction also tended

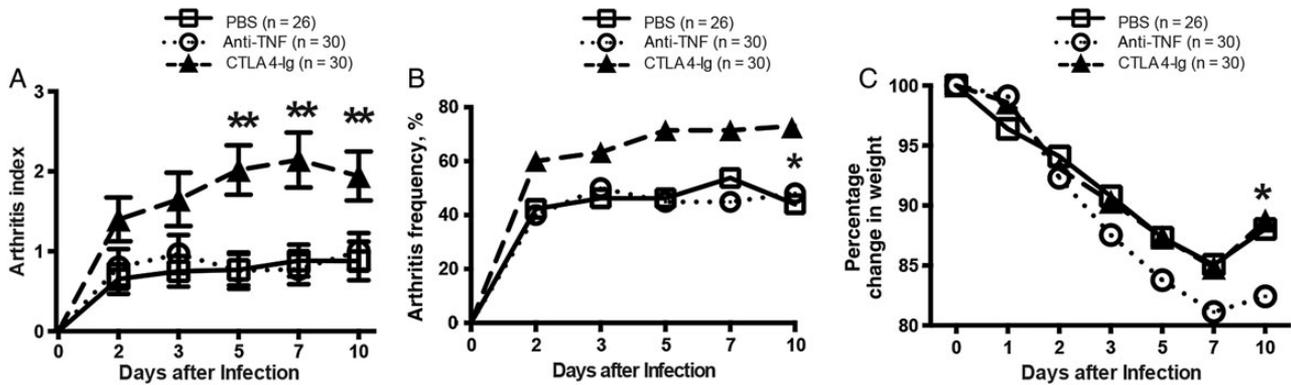


Figure 1. The effect of CTLA4 immunoglobulin (Ig) and anti-tumor necrosis factor (TNF) therapy on the clinical course of staphylococcal arthritis. NMRI mice inoculated with *Staphylococcus aureus* Newman strain ($1.1\text{--}1.7 \times 10^6$ colony-forming units/mouse) were treated with abatacept (CTLA4-Ig; 0.25 mg/g of body weight; $n = 30$), etanercept (anti-TNF; 5 $\mu\text{g/g}$ of body weight; $n = 30$), or phosphate-buffered saline (PBS; $n = 26$) twice weekly starting on day 7 before inoculation with bacteria and continuing until the animals were euthanized, on day 10. *A* and *B*, The frequency (*A*) and severity (*B*) of clinical arthritis in the mice were observed for 10 days after infection. *C*, The percentage changes in body weight from day 0 were also registered. The data from 3 independent experiments were pooled. Statistical evaluations were performed using the Mann-Whitney *U* test and the χ^2 test. Data are mean values \pm standard error of the mean. * $P < .05$ and ** $P < .01$.

to be enhanced in CTLA4-Ig-treated mice, compared with the control group (Figure 2C).

Anti-TNF Treatment Deteriorates Bacterial Clearance in the Bloodstream but Not in Joints

There were macroscopically more abscesses in kidneys from anti-TNF-treated mice ($P < .01$; Figure 3A) but not in those from CTLA4-Ig-treated mice, compared with PBS-treated mice. The abscess score and the actual bacterial load in the kidneys correlated significantly ($r = 0.59$; $P < .0001$), and the anti-TNF-treated mice had a >30-fold higher bacterial load in the kidneys, compared with the PBS-treated controls ($P = .004$; Figure 3B). This strongly suggests that anti-TNF treatment deteriorates the systemic bacterial clearance capacity.

To elucidate whether the deleterious effect on septic arthritis by CTLA4-Ig is due to poor bacterial clearance in local joints, bacterial counts in the joints were analyzed on day 5, when the clinical difference in arthritis severity became evident (Figure 3C). Joint CFU counts were positive in almost 90% of animals. Despite the greater frequency of clinical arthritis in CTLA4-Ig-treated mice (50% vs 30%), bacterial loads in the joints were similar between CTLA4-Ig-treated mice and PBS-treated mice. Intriguingly, anti-TNF-treated mice had significantly lower bacterial counts in the joints than PBS-treated mice, suggesting that neither anti-TNF nor CTLA4-Ig treatment increases the bacterial load locally in the joints.

CTLA4-Ig and Anti-TNF Therapies Induce Different Cytokine Profiles in Septic Arthritis Mice In Vivo

To investigate the systemic inflammatory response, we measured the serum levels of 7 cytokines (Figure 4A–G). The etanercept-bound TNF- α was known to be able to bind to the antibodies

used in the ELISA, although the biological activities of TNF- α were neutralized [18]. Intriguingly, TNF- α levels were significantly higher in mice that received anti-TNF therapy, compared with the controls ($P = .03$). Significantly lower interleukin 4 (IL-4) levels were detected in serum specimens from CTLA4-Ig-treated mice, compared with serum specimens from PBS-treated mice ($P = .0004$), and interleukin 2 (IL-2) levels also tended to be lower in the CTLA4-Ig-treated mice ($P = .07$). Despite more-severe bone destruction, mice that received CTLA4-Ig had the lowest serum levels of RANKL (Figure 4H), suggesting a potential inhibitory effect of CTLA4-Ig on RANKL production. No difference between the different treatment groups was observed with regard to other cytokines (Figure 4).

CTLA4-Ig Treatment In Vivo Reduces iNOS and Arginase-1 Expression in Peritoneal Macrophages

Three days after intraperitoneal exposure to heat-killed *S. aureus*, peritoneal leukocytes were composed of macrophages (23%–30%), neutrophils (8%–12%), and monocytes and lymphocytes (data not shown). Intriguingly, both iNOS intensity (an M1 macrophage marker) and arginase-1 intensity (an M2 macrophage marker) were lower in macrophages from CTLA4-Ig-treated mice, compared with intensities in anti-TNF-treated mice ($P < .05$; Figure 5A and 5B), indicating possible functional differences between macrophages in mice from these 2 treatment groups. TNF- α and IL-6 levels in peritoneal lavage tended to be lower in the CTLA4-Ig group, compared with the other 2 groups, on day 1 (Figure 5C).

DISCUSSION

In this study, we demonstrated that pretreatment with CTLA4-Ig significantly increased the frequency and severity of *S. aureus*

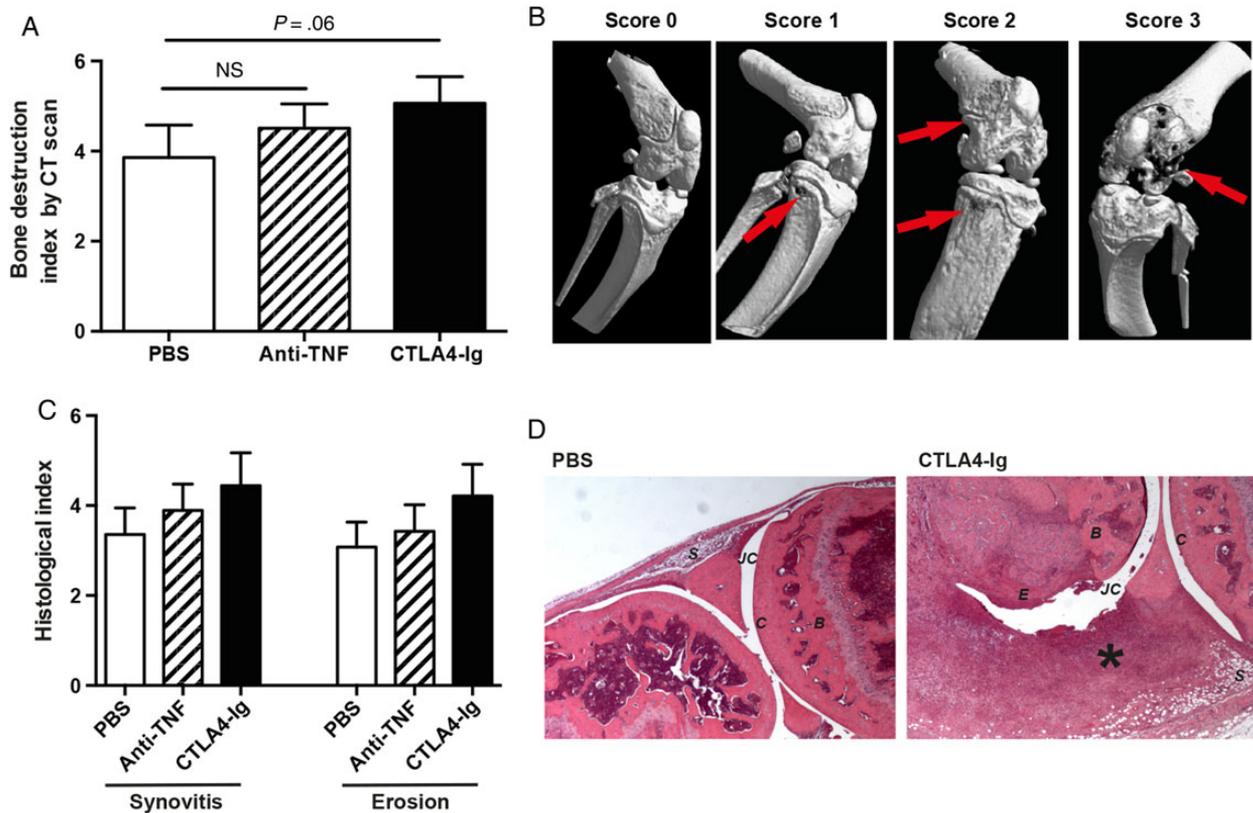


Figure 2. More-severe bone destruction was found in mice receiving CTLA4 immunoglobulin (Ig) treatment. The 3-dimensional joint structures and microscopic arthritis signs were semiquantitatively assessed by 2 observers who were blind to the treatment groups, using a scoring system from 0 to 3. *A*, Cumulative bone destruction scores of the joints from all 4 limbs of NMRI mice, based on microcomputed tomography. *B*, Representative computed tomographic images showing an intact knee joint (score 0), a knee joint with mild bone destruction on the proximal tibia (score 1; arrow), a knee joint with moderate bone erosion on the proximal tibia and the distal femur (score 2; arrows), and a heavily destructed knee joint on the distal femur (score 3; arrow). *C*, Histological evaluation of the joints from all 4 limbs 10 days after infection. *D*, Micrographs of histologically intact knee joints from a NMRI mouse inoculated with *Staphylococcus aureus* Newman strain that was treated with phosphate-buffered saline (PBS; left) and a heavily inflamed knee joint with severe bone and cartilage destruction from a NMRI mouse with septic arthritis treated with CTLA4-Ig (right). Hematoxylin and eosin stain was used. Original magnification $\times 10$. The asterisk indicates a heavily inflamed synovium. Statistical evaluations were performed using the Mann–Whitney *U* test. Data are presented as mean values \pm standard errors of the mean. Abbreviations: B, bone; C, cartilage; E, erosion of bone and cartilage; JC, joint cavity; NS, not significant; S, synovial tissue; TNF, tumor necrosis factor.

septic arthritis in mice, whereas the bacterial clearance remained unaffected. In contrast, mice pretreated with anti-TNF had no increased susceptibility to septic arthritis but had an impaired ability to clear *S. aureus* from the bloodstream.

Recently, a head-to-head study between abatacept (CTLA4-Ig) and adalimumab (anti-TNF- α) in patients with rheumatoid arthritis revealed that both biologics had a similar efficacy in reducing joint flares, but the CTLA4-Ig-treated group had fewer cases of serious infection, compared with the anti-TNF-treated group [4]. CTLA4-Ig had an acceptable safety profile and was well tolerated in patients with rheumatoid arthritis, with a marginal increase of serious infections, compared with placebo recipients [19], whereas patients with rheumatoid arthritis receiving anti-TNF therapy were shown to have an increased risk of some serious infections [20–22]. Indeed, the present study provides compelling evidence that anti-TNF therapy

deteriorates the ability of the host defense to eliminate *S. aureus*, since the bacterial load in the kidneys of mice treated with anti-TNF was 30 times higher than that in other groups. Also, the greater weight loss observed in anti-TNF-pretreated mice suggests more-severe *S. aureus* infection with increased bacterial load and renal abscesses. Of interest, patients with rheumatoid arthritis treated with anti-TNF often report weight gain, rather than weight loss [23], but this is probably due to the suppression of appetite caused by TNF. Neither the severity nor the frequency of septic arthritis were increased in mice receiving anti-TNF therapy, suggesting that deteriorated systemic bacterial killing capacity of the host does not necessarily lead to higher susceptibility to *S. aureus* septic arthritis. Surprisingly, fewer bacteria were found in the joints from mice treated with anti-TNF, compared with PBS-treated controls, whereas no tangible difference was detectable with regard to the arthritis severity.

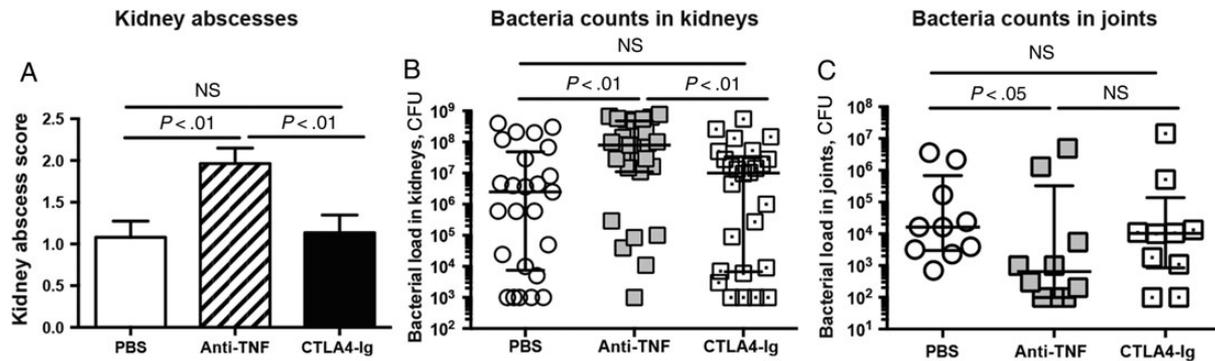


Figure 3. Treatment with anti-tumor necrosis factor (TNF) led to more-severe kidney abscesses and higher *Staphylococcus aureus* loads in kidneys. NMRI mice inoculated with *S. aureus* Newman strain ($1.1\text{--}1.7 \times 10^6$ colony-forming units [CFU]/mouse) were treated with abatacept (CTLA4 immunoglobulin [Ig]; 0.25 mg/g of body weight), etanercept (anti-TNF therapy; 5 μ g/g of body weight), or phosphate-buffered saline (PBS) twice weekly starting on day 7 before inoculation with bacteria and continuing until the animals were euthanized, on day 5 and day 10. *A* and *B*, Abscess scores of the kidneys from the mice euthanized 10 days after infection (*A*) and persistence of *S. aureus* in kidneys of the mice (*B*). The data from 3 independent experiments were pooled ($n = 25\text{--}27$ mice/group). *C*, Persistence of *S. aureus* in joints, including wrists, ankles, and knees of the mice (10/group) euthanized 5 days after infection. Statistical evaluations were performed using the Mann-Whitney *U* test. Data are presented as mean values \pm standard errors of the mean, for kidney abscesses, or median values with interquartile ranges, for bacterial load in kidneys. * $P < .05$ and ** $P < .01$. Abbreviation: NS, not significant.

The underlying mechanism associated with the discrepant bacteria loads in local joints and kidneys remains elusive. However, our data indicate that the severity of *S. aureus* septic arthritis is not exclusively linked to the amount of bacteria present in the joints but might also be determined by other factors, including proinflammatory cytokines and the extent of leukocyte infiltration in the synovium.

Both clinical signs and radiological changes of septic arthritis were aggravated in mice receiving CTLA4-Ig pretreatment, indicating that CTLA4-Ig therapy might increase the susceptibility of patients to *S. aureus* arthritis. Intriguingly, the number of invading *S. aureus* in the joints was unaltered by CTLA4-Ig treatment, which strongly suggests that enhanced joint inflammation in response to the presence of *S. aureus* in the joints, rather than poor bacterial control locally in the joints, is a more rational explanation for our observation. It is known that CD4⁺ T cells play a central role in *S. aureus* arthritis in mice, and depletion of CD4⁺ T cells ameliorated the severity of *S. aureus* septic arthritis [8, 9]. Regulatory T cells (Tregs) are potent suppressors of inflammatory responses, and they regulate autoimmunity by suppressing T-cell activation [24]. CD28 signaling has been shown to essentially control thymic development and peripheral homeostasis of regulatory T cells [25], and both B7-deficient mice and CD28-deficient mice had a profound decrease in immunoregulatory CD4⁺CD25⁺ T-cell counts [11]. As a result of decreased Treg counts, CTLA4-Ig transgenic mice displayed exacerbated spontaneous autoimmune diabetes [10], and CTLA4-Ig treatment accelerated rejection in a major histocompatibility complex class II mismatch transplant model [12]. Hence, we speculate that in our study, CTLA4-Ig treatment blunted Treg-associated antiinflammatory

function, resulting in exaggerated joint inflammation in response to invading *S. aureus*.

CTLA-4 is known to downregulate T-helper type 2 (Th2) differentiation [26]. IL-4, a Th2 cytokine, was significantly downregulated in mice with septic arthritis that received CTLA4-Ig, compared with controls. Intriguingly, IL-4 deficiency was previously shown to increase the severity of arthritis in experimental group B *Streptococcus* infection [27], which is in line with our results. IL-4 is known to inhibit classical activation of macrophages into M1 cells and to promote alternative activation of macrophages into M2 cells [28]. Tarkowski et al demonstrated that macrophages have a deteriorative effect in *S. aureus* septic arthritis with regard to the severity of arthritis lesions [29]. The significantly lower levels of IL-4 might indicate that there were more activated M1 cells that were responsible for deteriorated septic arthritis in the CTLA4-Ig group, compared with the control group. Notably, no significant differences were found in serum levels of IFN- γ and IL-2 (a Th1 cytokine) between the CTLA4-Ig group and the control group, suggesting that CTLA4-Ig therapy mainly affected the Th2 response. In agreement with the cytokine data, peritoneal macrophages exposed to heat-killed *S. aureus* exhibited lower expression of both iNOS (M1) and arginase-1 (M2) in mice receiving CTLA4-Ig treatment than in the anti-TNF group. In contrast to proinflammatory and antimicrobial M1 macrophage responses, M2 macrophages have antiinflammatory activity and play potent roles in wound healing and fibrosis by producing growth factors, including transforming growth factor β 1 (TGF- β 1) [30]. TGF- β 1 was shown to protect against collateral damage caused by the immune system by selectively promoting the apoptosis of effector CD8⁺ T cells in listeria infections [31]. Therefore, downregulation

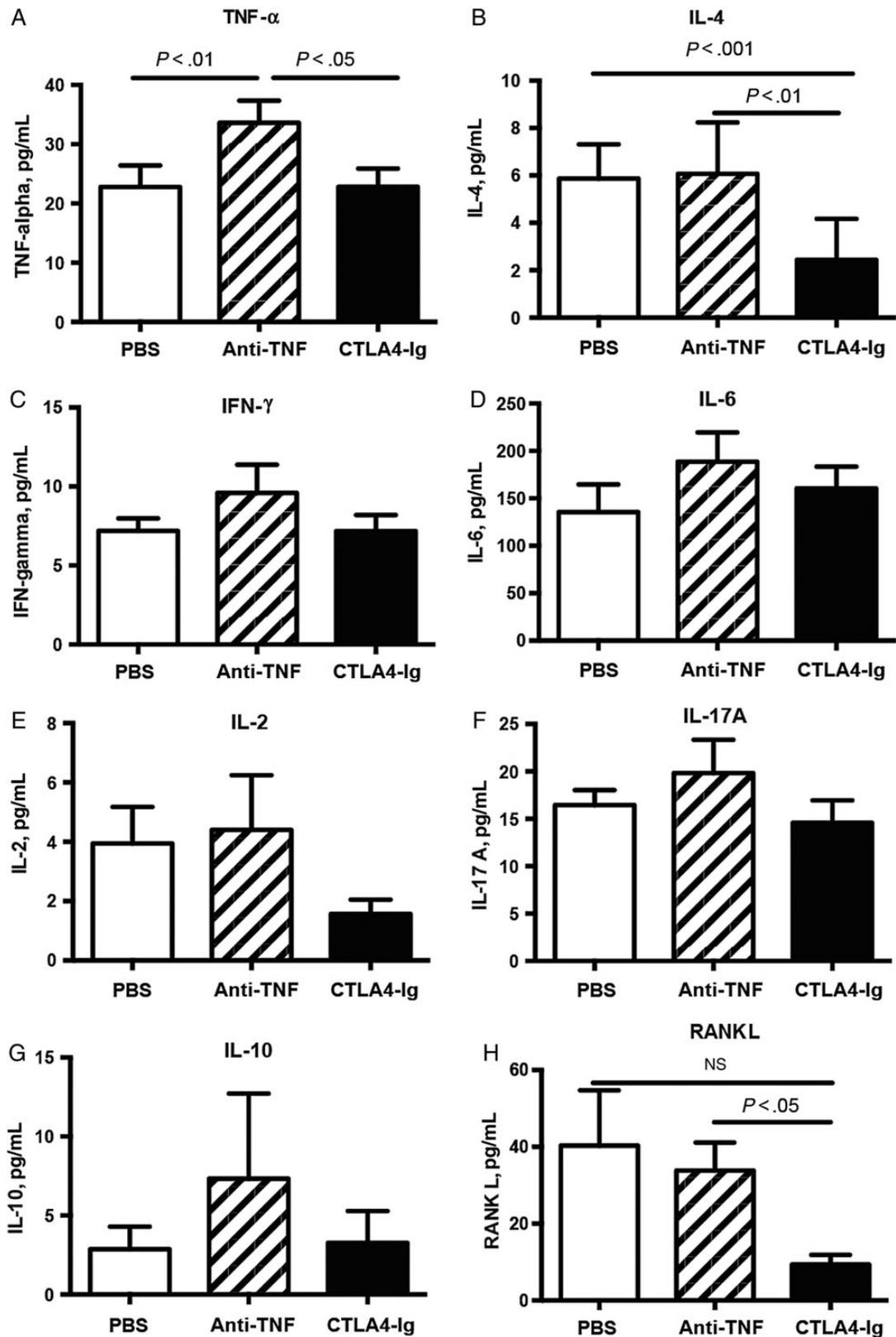


Figure 4. CTLA4 immunoglobulin (Ig) and anti-tumor necrosis factor (TNF) therapy before treatment resulted in different serum cytokine profiles of mice inoculated with *Staphylococcus aureus*. Serum levels of TNF- α , interleukin 4 (IL-4), interferon γ (IFN- γ), interleukin 6 (IL-6), interleukin 2 (IL-2), interleukin 17A (IL-17A), interleukin 10 (IL-10), and receptor activator of nuclear factor κ -B ligand (RANKL) were determined after termination of the experiment on day 10 after infection. Statistical evaluations were performed using the Mann-Whitney *U* test. Data are mean values \pm standard errors of the mean. Abbreviations: NS, not significant; PBS, phosphate-buffered saline.

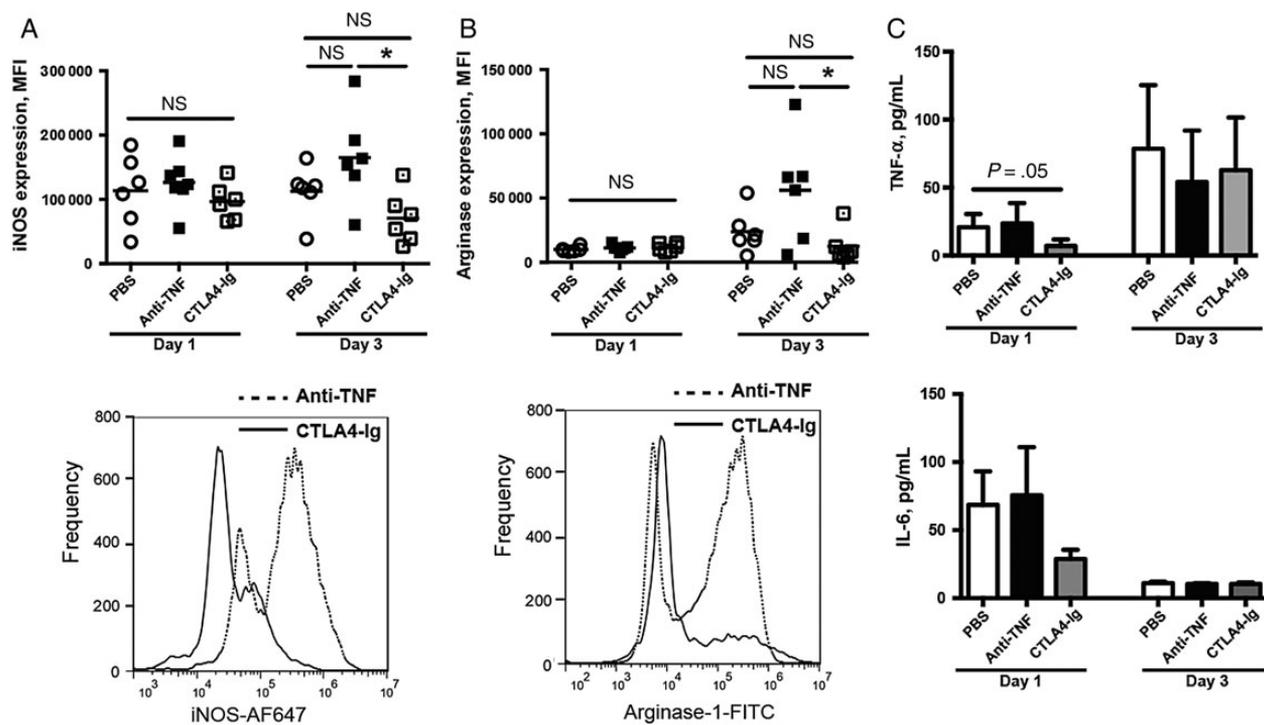


Figure 5. Proportion and characterization of intraperitoneal leukocytes. NMRI mice treated subcutaneously with etanercept ($n = 12$), abatacept ($n = 12$), or phosphate-buffered saline (PBS; $n = 13$) for 7 days were intraperitoneally injected with heat-killed *S. aureus* Newman strain (1×10^9 dead bacteria) in 200 μL of PBS. *A* and *B*, Quantitative median fluorescence intensity (MFI) graphs (upper panel) and histograms (lower panel) for iNOS expression (*A*) and arginase-1 expression (*B*) in peritoneal macrophages, analyzed by flow cytometry. *C*, Tumor necrosis factor α (TNF- α) levels (upper panel) and interleukin 6 (IL-6) levels (lower panel) in peritoneal lavage. Data are mean values \pm standard errors of the mean. * $P < .05$. Abbreviation: NS, not significant.

of M2 macrophages by CTLA4-Ig may contribute to an exaggerated immune response and significantly increase the joint damage in our setting.

Immunomodulating drugs may aggravate an infection, since they hamper the host defense against pathogens. However, they can also be used in infectious diseases such as septic arthritis [32] and meningitis [33] to downregulate the exaggerated inflammatory response that causes the tissue damage. Our earlier results suggest that, via TNF receptor 1, antibiotic-killed *S. aureus* causes long-lasting joint inflammation that might lead to postinfectious complications of *S. aureus* septic arthritis [34]. The combination of antibiotics and anti-TNF therapy was able to minimize postinfectious sequelae in mice with *S. aureus* septic arthritis [15], suggesting the use of an immunomodulatory adjuvant to an effective antibiotic as a new therapeutic strategy against *S. aureus* arthritis. Yet, deteriorated bacterial clearance in mice receiving anti-TNF therapy indicates the potential dangers associated with choosing inadequate antibiotics in combination with TNF inhibitors. Therefore, we seek other immune modulating medicines with lower infection risk profiles to downregulate the postinfectious inflammation in septic arthritis. In this study, CTLA4-Ig displayed an efficacy similar to that of anti-TNF therapy in controlling exaggerated joint inflammation when administered in combination with

an effective antibiotic (Supplementary Materials). The absence of deterioration in the clearance of *S. aureus* in the CTLA4-Ig treatment group implies that CTLA4-Ig might be superior to anti-TNF, owing to its lower infection risk profile.

Since the pharmacokinetics of biologics in humans and rodents differ, the doses of abatacept and etanercept used in this study might not be comparable to the doses used in patients. However, the abatacept and etanercept doses used here have been shown to exert full biologic functions in mice [15, 16]. Our data suggest that patients receiving anti-TNF therapy may experience deterioration in the clearance of *S. aureus*, while patients receiving CTLA4-Ig treatment might have a higher risk of developing more-severe symptoms of septic arthritis. Thus, the risk of site-specific staphylococcal infections in certain patients, such as those with *S. aureus* nasal carriage [35] and those undergoing hemodialysis or peritoneal dialysis [36], may need to be taken into account when choosing appropriate immunomodulatory therapies.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary

data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgment. We thank Prof Esbjörn Telemo for the critical reading of the manuscript.

Financial support. This work was supported by the Swedish Medical Research Council, the Swedish Medical Society, the Stiftelsen Clas Groschinsky Minnesfond, Scandinavian Society for Antimicrobial Chemotherapy Foundation, the Ingabritt and Arne Lundberg Foundation, the Swedish Association against Rheumatism, the Professor Nanna Svartz Foundation, and the University of Gothenburg.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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