

Protection with Antibody to Tumor Necrosis Factor Differs with Similarly Lethal *Escherichia coli* versus *Staphylococcus aureus* Pneumonia in Rats

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Background: Differing factors may alter the effects of antibody to tumor necrosis factor (TNF) in infection and sepsis. The authors tested whether bacteria type or treatment route alters antibody to TNF in a rat model of bacterial pneumonia.

Methods: Rats (n = 231) received similarly lethal doses of either intratracheal *Escherichia coli* or *Staphylococcus aureus* followed by treatment with either intratracheal or intraperitoneal antibody to TNF or control serum. Animals received antibiotics (cefotiam daily dose, 100 mg/kg) starting 4 h after inoculation and were studied for up to 96 h.

Results: Compared with *S. aureus*, *E. coli* increased serum TNF and interleukin-6 concentrations, lung lavage TNF concentrations, neutrophil counts, and alveolar-to-arterial oxygen gradients and decreased circulating neutrophils and lymphocytes ($P \geq 0.05$ for all). Compared with controls, with both bacteria, except for lung lavage TNF concentrations (which decreased with intratracheal but not with intraperitoneal antibody to TNF), treatment route did not alter the effects of antibody to TNF on any parameter ($P =$ not significant for all). Antibody to TNF reduced mortality rates (relative risk of death \pm SEM) with both *E. coli* (-1.6 ± 0.6 ; $P = 0.006$) and *S. aureus* (-0.5 ± 0.04 ; $P = 0.185$), but these reductions were greater with *E. coli* than with *S. aureus* in a trend approaching statistical significance ($P = 0.09$). Compared with controls, similarly ($P =$ not significant) with both bacteria, antibody to TNF decreased lung lavage and tissue bacteria concentrations (both $P < 0.05$) and serum TNF concentration ($P < 0.09$) and increased circulating neutrophils and lymphocytes (both $P \leq 0.01$). Compared with *S. aureus*, with *E. coli* antibody to TNF decreased alveolar-to-arterial oxygen gradients ($P = 0.04$) and increased serum interleukin-6 concentrations ($P = 0.003$).

Conclusion: Antibody to TNF improved host defense and survival rates with both lethal *E. coli* and *S. aureus* pneumonia, but protection was greater with *E. coli*, where TNF concentrations were higher than with *S. aureus*. The efficacy of antiinflammatory agents in sepsis may be altered by bacteria type.

AN excessive host inflammatory response has been closely associated with the organ injury and lethality related to sepsis and septic shock.¹ Of the mediators

activated and released during this response, tumor necrosis factor (TNF) was one of the first to be directly associated with these pathogenetic events during sepsis.² On the basis of this, over the past decade several different agents were developed to specifically inhibit TNF in patients with sepsis. Although these therapies were very beneficial in published animal models of sepsis,²⁻²⁵ none showed individual benefit in 10 clinical trials enrolling more than 5,000 patients.²⁶⁻³⁷ A review of this experience however shows that although these agents were tested almost exclusively in animal models using Gram-negative infectious challenges, patients in clinical trials presented with sepsis related to differing types of underlying infection, including Gram-negative, Gram-positive, and mixed bacterial infections.²⁻³⁷

Thus, variability in the release or activity of TNF with differing types of bacteria might provide one basis for the disparate effects antibody to TNF agents had in comparing preclinical and clinical trials.³⁸ Although TNF may be a central host mediator in tissue injury during Gram-negative bacterial infection, other mediators may play a more important role with other bacteria types. In several different studies, the TNF response with Gram-positive bacterial challenge has been shown to be either diminished or retarded when compared with Gram-negative bacterial challenges.³⁹⁻⁴¹ Consistent with this possibility, we showed before that similarly lethal doses of *Escherichia coli* and *Staphylococcus aureus* administered intratracheally in rats resulted in significantly greater TNF concentrations with the former than with the latter bacteria type.⁴² Furthermore, administration of granulocyte colony-stimulating factor to augment host defense function with these challenges, although increasing circulating neutrophils and survival rates with *S. aureus*, caused a paradoxical reduction in circulating neutrophils and worsened lung injury and survival rates with *E. coli*. Thus, in this preclinical model, stimulating the neutrophil, a cellular component in the inflammatory response, had very different effects comparing similarly lethal *E. coli* and *S. aureus* pneumonia. This differential effect of granulocyte colony-stimulating factor with Gram-positive bacterial pneumonia has now been observed in clinical trials testing this agent.⁴³

We hypothesized that differences in the contribution of TNF to the organ injury and lethality occurring with *E. coli* and *S. aureus* in our rat pneumonia model would result in differences in the effect of antibody to TNF

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Table 1. Number of Animals Receiving *Escherichia coli* or *Staphylococcus aureus* Challenge and Treated with Placebo (Control) or Intraperitoneal or Intratracheal Antibody to TNF Serum

Treatment	Type of Bacterial Challenge	
	<i>E. coli</i>	<i>S. aureus</i>
Control	38	40
IP antibody to TNF	35	41
IT antibody to TNF	37	40

IP = intraperitoneal; IT = intratracheal; TNF = tumor necrosis factor.

treatment with these two bacteria types. To test this, we challenged rats with similarly lethal intratracheal doses of either *E. coli* or *S. aureus* and then treated them with TNF antiserum. In addition, to compare the effects of administering antibody to TNF either at the site of infection or systemically, animals were randomized to receive either intratracheal or intraperitoneal treatment. All animals received antibiotic treatment in these studies to simulate conditions used previously in this model and those encountered in clinical trials. Our results suggest that with similarly lethal doses of bacteria in this model, antibody to TNF regardless of route of administration is more protective with *E. coli* than with *S. aureus* pneumonia.

Materials and Methods

Animal Care and Use

The Animal Care Committee (Weimar, Germany) approved the experimental protocol for this study. Up to four animals were maintained in a cage. Animals had free access to food and water throughout the study.

Study Design

Male Wistar rats ($n = 231$) weighing 250–300 g were randomized to receive an intratracheal inoculation with either *E. coli* or *S. aureus*. At the time of bacterial inoculation, animals were further randomized to receive in 0.3-ml volumes of antibody to TNF intratracheally and control serum intraperitoneally, control serum intratracheally and antibody to TNF intraperitoneally, or control serum in both compartments (table 1). Cefotiam (daily dose, 100 mg/kg) (Spizef; Takeda Pharma, Aachen, Germany) was given 4 h after bacterial inoculation and continued twice daily for 4 days. Animals were observed up to 96 h after bacterial inoculation. Either before or after inoculation, some animals were randomly selected to have either lung analysis (bacteria, leukocyte count, or TNF concentration) after death (6, 24, or 96 h after inoculation; 18–20 rats per study group) or serial circulating blood analysis (bacteria, leukocyte count, oxygen concentration, and TNF concentration) (before and 2, 6, 24, and 96 h after inoculation; 20–22 rats per study group).

Bacterial Inoculation

Frozen aliquots of either *E. coli* (American Type Culture Collection 25922) or *S. aureus* (American Type Culture Collection 25923) were thawed and inoculated into 250 ml brain–heart broth (Gibco, Paisley, Great Britain). After an incubation period of 5 h, the suspensions were centrifuged at 4°C and washed twice in phosphate-buffered saline. The final suspensions, as estimated by turbidimetry and compared with a predetermined standard curve, were prepared to produce a concentration of 2×10^9 colony-forming unit (CFU)/ml *E. coli* or 6×10^9 CFU/ml *S. aureus*. The concentrations were confirmed by plating serial dilutions on the appropriate culture medium and counting colonies. Animals were then given ketamine (100 mg/kg) (Ketanest; Parke–Davis, Berlin, Germany) and xylazine (8 mg/kg) (Rompun; Bayer, Leverkusen, Germany) intramuscularly. Following preoxygenation with 100% oxygen for 2 min via a face–shoulder mask, the trachea was visualized with a modified size 0 laryngoscope. An 18-gauge arterial catheter was inserted into the trachea, and 0.2 ml bacterial suspension (1.6×10^9 CFU/kg body weight *E. coli* and 4.8×10^9 CFU/kg body weight *S. aureus*) mixed with 0.3 ml treatment serum (antibody to TNF or control serum) was instilled, and the catheter was withdrawn. The animals were then placed in an oxygen chamber for 5 min and returned to their cages. Microbiologic testing before the study showed that both *E. coli* and *S. aureus* strains were susceptible to cefotiam, the antibiotic used in the study. The doses of *E. coli* and *S. aureus* and of antibiotic administered to animals in these studies had been shown previously to produce similar lethality rates.⁴²

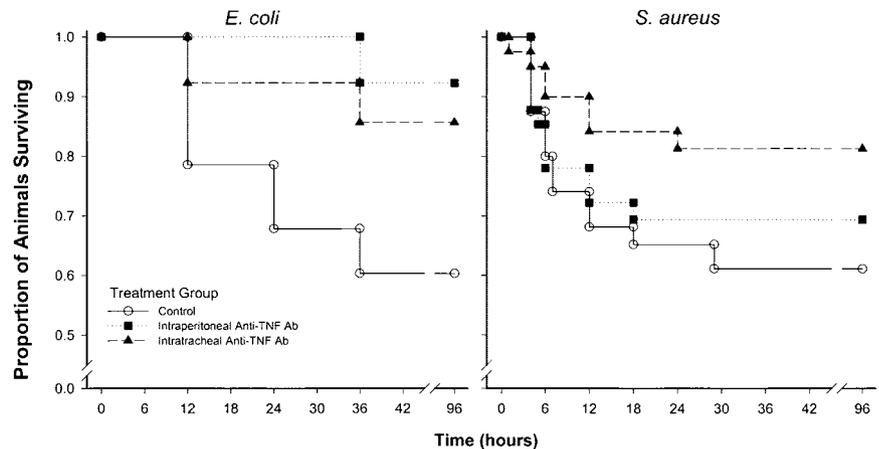
Antibody to TNF and Control Serum

The antibody to TNF and control sera used in this study were provided by Thomas Hartung, M.D., Ph.D. (Faculty of Biology, University of Konstanz, Germany). The antibody to TNF serum contained a polyclonal antibody raised in sheep to murine TNF- α . The activity was such that 1 ml antibody to TNF serum neutralized 1 μ g murine TNF- α . Control serum was obtained from sheep cared for under similar conditions but not immunized.

Lung Analysis

For lung analysis, tissue bacteria cultures and lavage cell, bacteria, and TNF concentrations were determined. Animals received isoflurane, 1.5%, and 100% oxygen via a mask at 6, 24, or 96 h. After blood sampling (see below), anesthetized animals were killed by cervical dislocation, and using sterile technique, the right lower lobe of the lung was removed and placed in 5 ml phosphate-buffered saline. The lung lobe was weighed and then homogenized, and 100 μ l homogenate was serially diluted in phosphate-buffered saline and then inoculated on agar plates for bacterial counts. The remaining lung

Fig. 1. Proportion of animals surviving following challenge with *Escherichia coli* or *Staphylococcus aureus* in controls (open circles) or animals treated with antibody to tumor necrosis factor (anti-TNF Ab) via the intratracheal (closed triangles) or intraperitoneal (closed squares) route. With both bacteria types, the effects of antibody to TNF on survival rates were similar ($P =$ not significant) when comparing intraperitoneal with intratracheal treatment with antibody to TNF.



was lavaged with 9 ml phosphate-buffered saline in 3-ml aliquots. The lavage fluid was collected. Of this fluid, a portion was used for quantitative bacteria cultures or determinations of TNF concentrations. The remainder was centrifuged, and the cell pellet was resuspended in phosphate-buffered saline. The number of cells in phosphate-buffered saline was counted with a hemocytometer, and a differential cell count was determined on a smear using Wright stain.

Blood Analysis

For circulating blood analysis, animals were anesthetized with isoflurane, 1.5–2%, in 100% oxygen *via* a face-shoulder mask, and tail artery blood was collected in heparinized syringes for determination of TNF- α serum concentrations (2, 6, 24, and 96 h) and leukocyte counts and arterial blood gas analysis (both before and at 6, 24, and 96 h). Total blood leukocytes were counted using a hemocytometer, and a differential cell count was determined on a blood smear using Wright stain. After arterial blood gas analysis, differences in alveolar-to-arterial oxygen tension were calculated using a standard formula. For quantitative bacteria culture of circulating blood from animals selected for lung analysis, after anesthesia and before sacrifice, blood was obtained using sterile technique *via* puncture of the abdominal aorta, and 100 μ l was inoculated on blood agar plates.

TNF and Interleukin-6 Determinations

To measure rat TNF- α concentrations in blood or lung lavage samples, a solution, 0.5%, of rabbit antibody to rat TNF- α serum was incubated in 96-well plates. After 24 h of incubation at 4°C and washing, standard and test serum (or lavage) fluids were added, and the plates were incubated for 4 h at room temperature. After washing, a 1 μ g/ml concentrated biotin-conjugated antibody to rat TNF (PharMingen, San Diego, CA) was added. After repeated washing, 0.5 μ g/ml concentrated streptavidin-peroxidase conjugate solution (Dianova, Hamburg, Germany) was added. After a further 30 min, the wells were

washed, and the peroxidase substrate 3,3',5,5'-tetramethylbenzidine (Sigma, Deisenhofen, Germany) was added. The reaction was stopped after 15 min with 1 M H₂SO₄, and the absorption of the solution was read at 450 nm. In some animals from each group, TNF- α bioactivity concentrations were determined in lung lavage samples at 6 h only for comparison with the immunoassay. All reported results are those of the immunoassay unless specified. This was done with the WEHI 164 cell bioactivity assay as previously described.⁴⁴ Serum interleukin-6 (IL-6) concentrations were determined using a commercial assay (Biosource, Camarillo, CA).

Statistics

Survival was assessed using a Cox proportional hazards model with terms for type of bacteria (*E. coli* or *S. aureus*), treatment (antibody to TNF or placebo), route of treatment (intratracheal or intraperitoneal), and the various interactions.⁴⁵ Treatment effects are presented as relative risk \pm SEM of the estimate. ANOVA was performed on all other parameters in an attempt to explain the difference in treatment effect between the two bacterial challenges.⁴⁶ This model included terms for type of bacteria, treatment, route of treatment, time, and the various interactions. Data are presented as mean \pm SEM. $P < 0.05$ was considered statistically significant; $P < 0.10$ was considered a trend. Data were transformed where appropriate for analysis.

Results

Clinical Manifestations and Survival

At 6 h after intratracheal inoculation with either *E. coli* or *S. aureus*, animals appeared weak and lethargic. In controls without antibody to TNF treatment, *E. coli* and *S. aureus* challenges produced similar mortality rates ($P =$ not significant [NS]) (fig. 1). Compared with control treatment, antibody to TNF had similar effects on mortality rates associated with intratracheal and intraperitoneal routes of treatment ($P =$ NS), and we com-

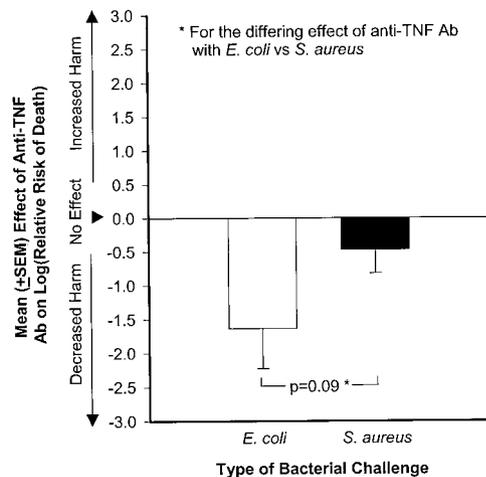


Fig. 2. Mean (\pm SEM) effect of antibody to tumor necrosis factor (anti-TNF Ab) on the log (relative risk of death) following challenge with *Escherichia coli* (open bar) or *Staphylococcus aureus* (closed bar). Antibody to TNF treatment decreased the relative risk of death with both bacteria, but this decrease was greater with *E. coli* than with *S. aureus*.

bin over this variable. Antibody to TNF reduced mortality rates with both *E. coli* and *S. aureus* (fig. 1). However, these reductions (relative risk of death \pm SEM) were greater with *E. coli* (-1.6 ± 0.6 ; $P = 0.006$) than with *S. aureus* (-0.5 ± 0.04 ; $P = 0.185$) in a trend that approached being statistically significant ($P = 0.09$ for the differing effects of antibody to TNF with *E. coli* vs. *S. aureus*) (fig. 2).

Lung Analysis

The effects of antibody to TNF on both lung and blood parameters, with the exception of lung lavage TNF concentrations, were not significantly different when comparing the differing time points or the two routes of antibody to TNF treatment studied ($P = \text{NS}$ for all). We therefore averaged across time and route of treatment for all parameters except lung lavage TNF concentrations to increase our ability to determine a cause for the beneficial effect of antibody to TNF on overall survival as well as for the possible advantage it showed with *E. coli* compared with *S. aureus*.

Compared with *S. aureus*, *E. coli* was associated with increased lung lavage TNF concentrations (5.9 ± 0.2 vs. 4.8 ± 0.39 log pg/ml, respectively; $P = 0.001$) and neutrophil counts (11.9 ± 1.0 vs. 5.0 ± 0.7 cells $\times 10^4$ /ml, respectively; $P = 0.0001$) and with fewer lung lavage bacteria (6.8 ± 0.8 vs. 9.2 ± 1.0 log CFU/ml, respectively; $P = 0.003$) and lung tissue bacteria (7.3 ± 0.5 vs. 8.8 ± 0.6 log CFU/g, respectively; $P = 0.05$) (table 2).

Similarly ($P = \text{NS}$) with both *E. coli* and *S. aureus*, compared with controls, intratracheal but not intraperitoneal antibody to TNF reduced lung lavage TNF concentrations, but these decreases were smaller over time ($P = 0.0001$) (table 2; fig. 3). At 6 h for the two bacteria combined, lung lavage TNF concentrations measured by immunoassay (9.1 ± 0.2 log pg/ml with control treatment vs. 8.8 ± 0.1 log pg/ml with intraperitoneal antibody to TNF vs. 5.6 ± 0.3 log pg/ml with intratracheal

Table 2. Serial Mean (\pm SEM) Lung Lavage TNF Concentrations and Bacteria, Neutrophil Macrophage, and Lung Tissue Bacteria Counts 6–96 h after *Escherichia coli* or *Staphylococcus aureus* Challenge in Animals Treated with Placebo (Control) or Intraperitoneal or Intratracheal Antibody to TNF Serum

Parameter/Treatment	Type of Bacterial Challenge					
	<i>E. coli</i>			<i>S. aureus</i>		
	6 h	24 h	96 h	6 h	24 h	96 h
BAL TNF (log $\mu\text{g}/\text{ml}$)						
Control	8.6 ± 0.2	6.9 ± 0.4	4.2 ± 0.4	10.0 ± 0.3	5.3 ± 0.3	2.9 ± 0.4
IP antibody to TNF	8.6 ± 0.2	6.1 ± 0.3	4.5 ± 0.4	9.2 ± 0.2	6.8 ± 0.3	2.7 ± 0.3
IT antibody to TNF	5.0 ± 0.1	4.1 ± 0.1	3.5 ± 0.1	7.1 ± 0.7	3.9 ± 0.2	2.8 ± 0.3
BAL bacteria (log CFU/ml)						
Control	11.2 ± 0.3	11.6 ± 3.9	12.6 ± 2.9	16.2 ± 0.3	10.7 ± 0.6	2.9 ± 0.7
IP antibody to TNF	11.6 ± 0.7	9.2 ± 3.1	9.0 ± 0.5	15.3 ± 0.3	10.1 ± 0.1	1.8 ± 0.5
IT antibody to TNF	–	6.0 ± 0.6	5.0 ± 1.4	14.7 ± 0.2	10.7 ± 0.7	1.2 ± 0.5
Lung tissue bacteria (log CFU/g)						
Control	7.5 ± 1.4	11.5 ± 1.2	7.2 ± 2.5	12.3 ± 0.3	13.7 ± 1.4	6.5 ± 1.4
IP antibody to TNF	4.7 ± 1.6	11.7 ± 0.7	5.4 ± 1.9	11.8 ± 0.3	12.6 ± 0.5	7.7 ± 1.5
IT antibody to TNF	8.1 ± 1.0	9.9 ± 1.0	1.1 ± 0.8	11.7 ± 0.6	12.6 ± 1.3	6.0 ± 1.2
BAL neutrophils (cells $\times 10^4/\text{ml}$)						
Control	17.8 ± 3.7	18.0 ± 3.2	2.3 ± 0.8	6.2 ± 2.1	6.7 ± 1.8	2.0 ± 0.4
IP antibody to TNF	13.2 ± 1.2	19.3 ± 4.6	3.0 ± 0.7	10.3 ± 2.0	9.7 ± 3.4	2.2 ± 0.4
IT antibody to TNF	14.1 ± 3.1	16.7 ± 2.2	3.0 ± 0.6	7.5 ± 2.2	9.9 ± 2.8	2.0 ± 0.3
BAL macrophages (cells $\times 10^4/\text{ml}$)						
Control	0.22 ± 0.03	–	0.58 ± 0.12	0.06 ± 0.03	0.47 ± 0.17	0.33 ± 0.05
IP antibody to TNF	0.22 ± 0.05	–	1.60 ± 0.06	0.22 ± 0.08	0.29 ± 0.15	1.15 ± 0.55
IT antibody to TNF	0.21 ± 0.06	–	0.55 ± 0.09	0.19 ± 0.53	0.99 ± 0.35	0.91 ± 0.20

BAL = bronchoalveolar lavage; CFU = colony-forming unit; IP = intraperitoneal; IT = intratracheal; TNF = tumor necrosis factor.

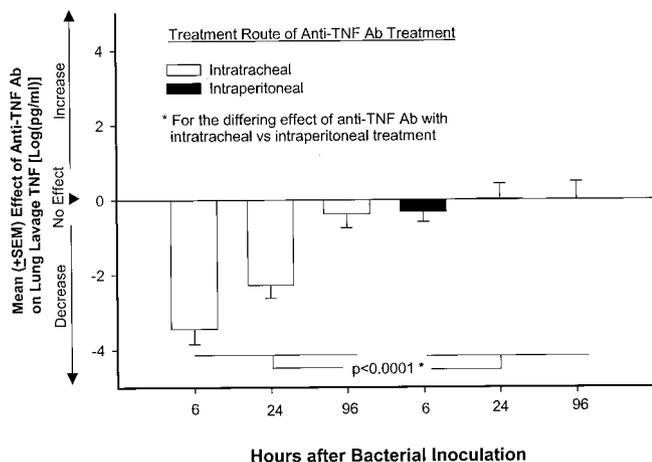
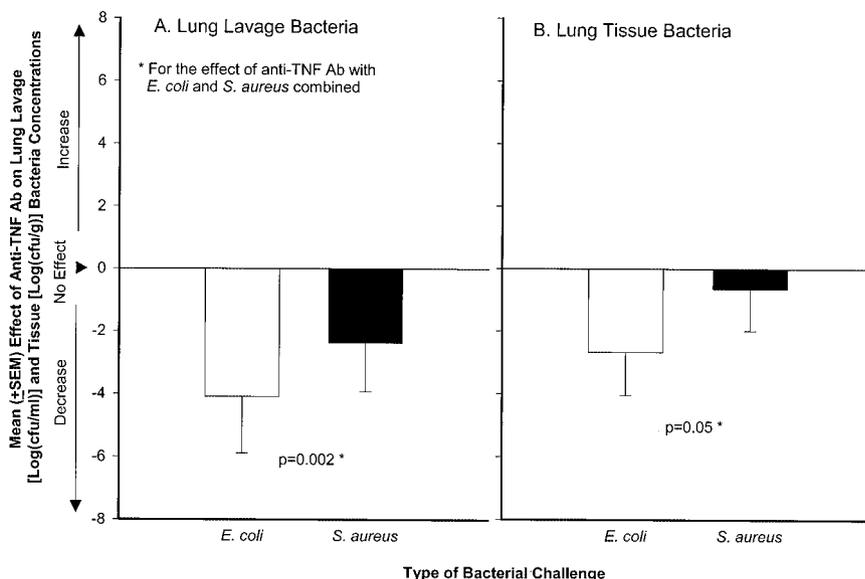


Fig. 3. Mean (\pm SEM) effects of intratracheal (open bars) or intraperitoneal (closed bars) antibody to tumor necrosis factor (anti-TNF Ab) on lung lavage TNF concentrations following challenge with *Escherichia coli* or *Staphylococcus aureus*. With both bacteria types, lung lavage TNF concentrations were reduced with intratracheal but not with intraperitoneal antibody to TNF, but these decreases were smaller over time.

antibody to TNF) were of the same magnitude and showed the same ordering by route of administration as TNF bioactivity concentrations (9.9 ± 0.1 log pg/ml with control treatment vs. 9.6 ± 0.1 log pg/ml with intraperitoneal antibody to TNF vs. 0 with intratracheal antibody to TNF (bioactivity concentrations were so reduced as to be nondetectable). Compared with control treatment, similarly ($P = NS$) with both bacteria types and routes of treatment, antibody to TNF was associated with reductions in bacteria in both lung lavage (9.6 ± 1.2 vs. 6.6 ± 0.7 log CFU/ml, respectively; $P = 0.002$) and lung tissue (9.2 ± 0.8 vs. 7.7 ± 0.5 log CFU/g, respectively; $P = 0.05$) samples (table 2; fig. 4). Other lung analysis measures did not differ ($P = NS$) when comparing bacteria types or treatments during the study.

Fig. 4. Mean (\pm SEM) effects of antibody to tumor necrosis factor (anti-TNF Ab) on lung lavage (A) and lung tissue bacteria (B) concentrations after challenge with *Escherichia coli* (open bar) or *Staphylococcus aureus* (closed bar). Similarly ($P =$ not significant) with both bacteria types, antibody to TNF decreased lung lavage and tissue bacteria concentrations.



Blood Analysis

Compared with *S. aureus*, *E. coli* increased serum TNF (5.8 ± 0.1 vs. 5.0 ± 0.1 log pg/ml, respectively; $P = 0.001$) and IL-6 (5.5 ± 0.4 vs. 3.9 ± 0.7 log pg/ml, respectively; $P = 0.02$) concentrations and alveolar-to-oxygen gradients (409 ± 8 vs. 373 ± 7 mmHg, respectively; $P = 0.001$) and decreased circulating neutrophils (1.3 ± 0.1 vs. 1.8 ± 0.1 cells $\times 10^3$ /mm³, respectively; $P = 0.0001$) and lymphocytes (2.9 ± 0.3 vs. 3.6 ± 0.2 cells $\times 10^3$ /mm³, respectively; $P = 0.0001$) (tables 3 and 4).

Compared with control treatment, similarly ($P = NS$) with both bacteria types and routes of treatment, antibody to TNF was associated with a trend toward reduction in serum TNF concentrations (5.5 ± 0.1 vs. 5.2 ± 0.1 log pg/ml, respectively; $P = 0.09$) and significant increases in circulating neutrophils (1.2 ± 0.1 vs. 1.7 ± 0.1 cells $\times 10^3$ /ml, respectively; $P = 0.01$) and lymphocytes (2.7 ± 0.2 vs. 3.6 ± 0.2 cells $\times 10^3$ /ml, respectively; $P = 0.001$) (tables 3 and 4; fig. 5). Differently for the two bacteria types, compared with controls, antibody to TNF reduced alveolar-to-arterial oxygen gradients more with *E. coli* than with *S. aureus* ($P = 0.04$) and increased serum IL-6 levels with *E. coli* but reduced them with *S. aureus* ($P = 0.003$) (table 3; fig. 6). Other parameters in blood analysis did not differ ($P = NS$) when comparing bacteria type or treatment during the study.

Discussion

Compared with *S. aureus*, similarly lethal *E. coli* doses in this rat model increased serum and lung lavage TNF concentrations, lung lavage neutrophil counts, and serum IL-6 concentrations and decreased arterial oxygenation (i.e., increased alveolar-to-oxygen gradients) and circulating neutrophil and lymphocyte counts. With the

Table 3. Serial Mean (\pm SEM) Blood Bacteria, Neutrophil, and Lymphocyte Counts and AaO₂ at Baseline (0 h) or 6–96 h after *Escherichia coli* or *Staphylococcus aureus* Challenge in Animals Treated with Placebo (Control) or Intraperitoneal or Intratracheal Antibody to TNF Serum

Parameter/Treatment	Type of Bacterial Challenge							
	<i>E. coli</i>				<i>S. aureus</i>			
	0 h	6 h	24 h	96 h	0 h	6 h	24 h	96 h
Bacteria (log CFU/ml)								
Control	–	0.6 \pm 0.6	3.5 \pm 1.7	0	–	1.2 \pm 0.7	2.9 \pm 0.5	1.9 \pm 0.5
IP antibody to TNF	–	0.4 \pm 0.4	5.8 \pm 2.8	0.5 \pm 0.5	–	0	4.7 \pm 2.3	1.7 \pm 0.6
IT antibody to TNF	–	0	2.9 \pm 1.9	0.8 \pm 0.5	–	0	2.6 \pm 1.6	1.3 \pm 0.5
Neutrophil (cells \times 10 ³ /mm ³)								
Control	0.9 \pm 0.1	0.6 \pm 0.1	1.4 \pm 0.3	2.1 \pm 0.5	0.7 \pm 0.1	0.5 \pm 0.1	2.1 \pm 0.3	2.3 \pm 0.9
IP antibody to TNF	0.8 \pm 0.1	0.6 \pm 0.1	2.1 \pm 0.4	2.1 \pm 0.5	0.7 \pm 0.1	0.9 \pm 0.2	2.7 \pm 0.5	2.3 \pm 0.5
IT antibody to TNF	1.1 \pm 0.2	0.6 \pm 0.1	2.7 \pm 0.3	1.6 \pm 0.6	0.8 \pm 0.1	0.9 \pm 0.2	2.8 \pm 0.6	2.7 \pm 0.6
Lymphocytes (cells \times 10 ³ /mm ³)								
Control	8.9 \pm 0.6	1.5 \pm 0.6	1.5 \pm 0.1	1.8 \pm 0.3	6.1 \pm 1.2	1.5 \pm 0.2	2.5 \pm 0.4	8.3 \pm 1.1
IP antibody to TNF	9.3 \pm 0.5	9.3 \pm 0.5	1.9 \pm 0.1	3.1 \pm 0.5	5.7 \pm 1.1	2.1 \pm 0.3	3.3 \pm 0.5	7.8 \pm 1.1
IT antibody to TNF	8.9 \pm 0.5	8.9 \pm 0.5	1.9 \pm 0.1	3.4 \pm 0.3	6.4 \pm 1.1	1.6 \pm 0.1	2.9 \pm 0.3	8.2 \pm 1.0
AaO ₂ (mmHg)								
Control	359 \pm 13	452 \pm 22	443 \pm 20	1.4 \pm 0.2	359 \pm 27	380 \pm 10	379 \pm 19	356 \pm 34
IP antibody to TNF	374 \pm 22	344 \pm 17	416 \pm 26	416 \pm 29	366 \pm 19	399 \pm 22	360 \pm 20	384 \pm 32
IT antibody to TNF	409 \pm 16	363 \pm 18	416 \pm 23	469 \pm 17	321 \pm 20	383 \pm 25	356 \pm 19	357 \pm 21

AaO₂ = alveolar-to-arterial oxygen gradient; CFU = colony-forming unit; IP = intraperitoneal; IT = intratracheal; TNF = tumor necrosis factor.

exception of lung lavage TNF concentrations, antibody to TNF had similar effects whether it was administered *via* intratracheal or intraperitoneal routes. Overall with both bacteria types, antibody to TNF was associated with decreased lung bacteria and serum and lung lavage TNF concentrations, increased circulating neutrophils and lymphocytes, and improved survival rates. Although not reaching statistical significance, there was a strong trend for improved survival rates to be greater with *E. coli* than with *S. aureus*. This difference in survival rates with antibody to TNF with *E. coli* compared with *S. aureus* was associated with bigger improvements in oxygenation and with increases in serum IL-6 concentrations.

Increased circulating neutrophils and lymphocytes and reduced lung bacteria counts with treatment during both *E. coli* and *S. aureus* pneumonia suggest that improved survival rates with antibody to TNF may have been related to better host defense. Decreased serum TNF concentrations with antibody to TNF may have reduced endothelial activation and leukocyte adhesion molecule expression in nonpulmonary tissues, increasing the availability of these host effector cells at the lung

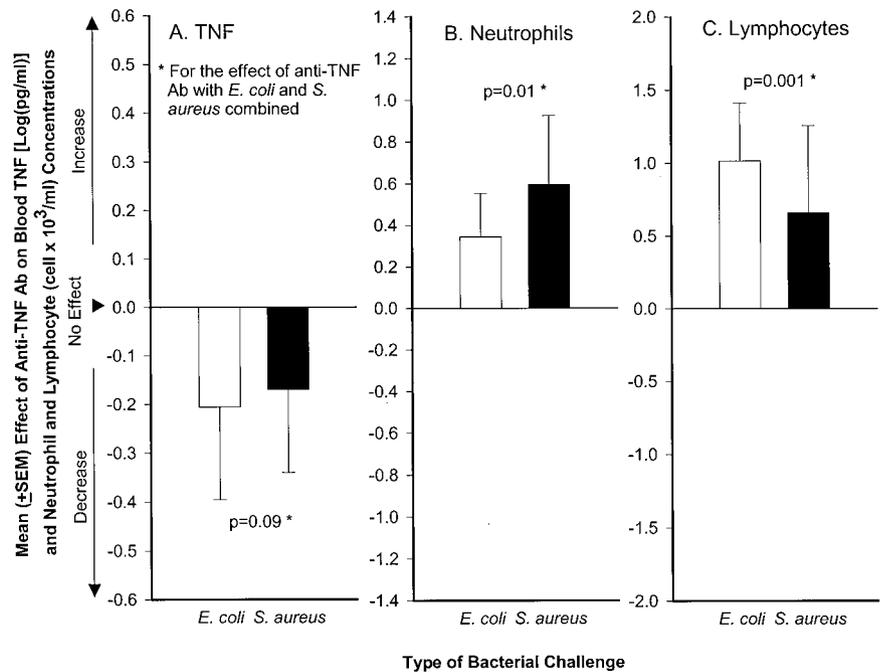
for microbial clearance.^{9,47,48} Although increases in leukocyte counts were not evident in lung lavage samples, increased numbers of effector cells may have been present in pulmonary interstitial spaces. Alternatively, however, the effects of antibody to TNF on circulating leukocytes and lung bacteria may represent independent protective mechanisms. Decreases in serum TNF concentrations and increased circulating leukocytes with antibody to TNF may reflect reductions in systemic vascular inflammatory injury and improved systemic hemodynamic function as has been shown in other models.^{49,50} In addition, decreases in lung TNF concentrations with antibody to TNF may have reduced local inflammatory responses that have been suggested to interfere with host defense.⁵¹ It is also possible that reductions in lung TNF concentrations removed a direct stimulus for bacterial growth as has been demonstrated in *in vitro* systems.^{52–54} This latter mechanism appears less likely since intratracheal treatment, which resulted in the greatest reductions in lung lavage TNF concentrations, did not have differing effects on lung bacteria counts or survival rates when compared with intraperitoneal treatment.

Table 4. Serial Mean (\pm SEM) Blood TNF Concentrations (log μ g/ml) 2–96 h after *Escherichia coli* or *Staphylococcus aureus* Challenge in Animals Treated with Placebo (Control) or Intraperitoneal or Intratracheal Antibody to TNF Serum

Treatment	Type of Bacterial Challenge							
	<i>E. coli</i>				<i>S. aureus</i>			
	2 h	6 h	24 h	96 h	2 h	6 h	24 h	96 h
Control	5.9 \pm 0.2	5.4 \pm 0.3	5.4 \pm 0.2	8.0 \pm 0.5	4.7 \pm 0.1	5.3 \pm 0.3	4.7 \pm 0.3	6.5 \pm 0.3
IP antibody to TNF	5.6 \pm 0.2	5.3 \pm 0.4	5.9 \pm 0.2	6.9 \pm 0.5	4.7 \pm 0.2	4.7 \pm 0.5	3.9 \pm 0.7	6.4 \pm 0.4
IT antibody to TNF	5.3 \pm 0.2	5.3 \pm 0.3	5.8 \pm 0.2	7.2 \pm 0.3	4.6 \pm 0.1	5.0 \pm 0.2	4.6 \pm 0.1	6.8 \pm 0.3

IP = intraperitoneal; IT = intratracheal; TNF = tumor necrosis factor.

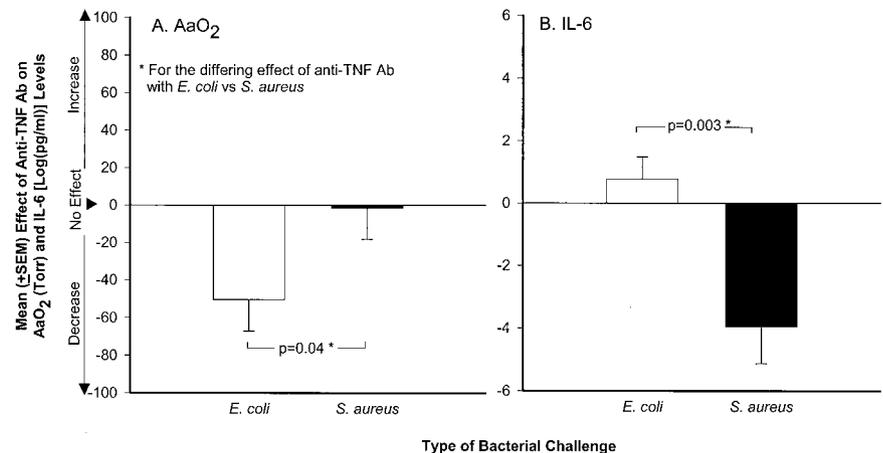
Fig. 5. Mean (\pm SEM) effects of antibody to tumor necrosis factor (*anti-TNF Ab*) on serum TNF concentrations (A) and circulating neutrophil (B) and lymphocyte (C) counts after challenge with *Escherichia coli* (open bar) or *Staphylococcus aureus* (closed bar). Similarly ($P =$ not significant) with both bacteria types, antibody to TNF decreased serum TNF concentrations and increased circulating neutrophils and lymphocytes.



Our results differ from those of other studies, which demonstrated that inhibition of TNF interferes with local host defense function and outcome with extravascular infection.^{4,55} These differences however may in part reflect variation in the underlying severity of infection studied. We have shown that independent of its type and route, the severity of an infectious challenge has an important influence on the effect of antiinflammatory agents in animal models of sepsis.⁵⁶ With mild infections (control mortality rates of less than 25%), antiinflammatory agents may interfere with local protective host mechanisms and worse outcome. However, at higher mortality rates, antiinflammatory agents become progressively more beneficial as the host loses its ability to contain an extravascular nidus of infection and the ensuing systemic inflammatory response results in injury in noninfected tissues. This influence of disease severity is demonstrable in clinical trials testing antiinflammatory agents in sepsis as well.^{37,57,58}

We hypothesized that antibody to TNF would be more efficacious with *E. coli* than with *S. aureus* in this pneumonia model based on prior studies showing that TNF levels were greater with the former than with the latter.⁴² Once again in this study, TNF concentrations with similarly lethal challenges were greater with *E. coli* than with *S. aureus*. With this, although not reaching a statistically significant level, there was a strong trend ($P = 0.09$) for antibody to TNF to improve survival rates more with *E. coli* than with *S. aureus*. The physiologic basis for this advantage may be related to the improved oxygenation observed with treatment during *E. coli* but not *S. aureus* infection. Alternatively, suppression of serum IL-6 concentrations with antibody to TNF during *S. aureus* but not *E. coli* infection may represent a maladaptive response, although not one sufficient to totally negate the beneficial survival effect associated with treatment. Of note, although not significant, reductions in lung bacteria counts were greater with *E. coli* than

Fig. 6. Mean (\pm SEM) effects of antibody to tumor necrosis factor (*anti-TNF Ab*) on alveolar-to-arterial oxygen gradients (AaO₂) (A) and serum interleukin-6 (IL-6) concentrations (B) after challenge with *Escherichia coli* (open bars) or *Staphylococcus aureus* (closed bars). Differently for the two bacteria types, antibody to TNF reduced AaO₂ more with *E. coli* than with *S. aureus* and increased serum IL-6 concentrations with *E. coli* but reduced them with *S. aureus*.



with *S. aureus*, suggesting that there may have been differential improvements in host defense with antibody to TNF when comparing the two infection types. Overall, however, these findings are consistent with other work demonstrating that the role of TNF in the pathogenesis of organ injury and outcome during lethal infection with differing bacterial types may not be the same.³⁸⁻⁴¹ Therefore, as this study suggests, type of bacterial infection may be an important determinant altering the effectiveness of mediator-specific antiinflammatory agents such as TNF antagonists in sepsis. We showed previously in this same model that neutrophil stimulation with granulocyte colony-stimulating factor greatly improved survival with *S. aureus* but worsened it with *E. coli*.⁴²

Studies suggest that injury to the alveolar-capillary membrane resulting in the intravascular uptake of TNF produced locally at a site of pneumonia may be an important pathogenetic step in the development of systemic inflammation and death.^{59,60} Injury related to the production of TNF itself has been implicated in the loss of this compartmentalized response.⁶¹ On the basis of this, we reasoned that inhibiting alveolar TNF directly would result in the greatest improvements in outcome in our model. However, despite significant decreases in lung lavage TNF concentrations with intratracheal but not with intraperitoneal antibody to TNF, route of treatment did not alter lung leukocyte counts, any parameter of outcome, or systemic TNF concentrations with either bacteria type. This finding is consistent with those of other studies suggesting that the local pulmonary production of TNF even during lethal pneumonia may not be necessary alone to cause either the lung injury or the systemic inflammatory response observed.⁶² Other mediators (e.g., IL-1 or IL-8) produced in the lung in addition to TNF may be sufficient for the extravascular recruitment of inflammatory cells and the lung injury that occurs. Furthermore, loss of compartmentalization with the release of either microbe and microbial toxins or host mediators other than TNF may be sufficient to produce the systemic inflammatory response associated with infection in this model.

In conclusion, although antibody to TNF improved survival with both lethal *E. coli* and *S. aureus* pneumonia in this rat model, this protective effect was stronger with *E. coli* than with *S. aureus*. These findings suggest that the efficacy of mediator-specific antiinflammatory agents in sepsis may relate in part to the type of underlying infection. Because Gram-positive bacterial infections are increasingly frequent in patients presenting with sepsis, our findings suggest that accounting for the type of bacterial infection may be important to improve the effectiveness of antiinflammatory therapies for these patients.

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