

## Endogenous Gamma Interferon, Tumor Necrosis Factor, and Interleukin-6 in *Staphylococcus aureus* Infection in Mice

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**The production and roles of endogenous gamma interferon (IFN- $\gamma$ ), tumor necrosis factor (TNF), and interleukin-6 (IL-6) in both lethal and nonlethal infections of *Staphylococcus aureus* were investigated in mice. In the case of nonlethal infection, although no bacteria were detected in the bloodstreams, bacteria that colonized and proliferated persistently for 3 weeks were found in the kidneys. All mice given lethal injections died within 7 days, and large numbers of bacteria were detected in the bloodstreams, spleens, and kidneys. The first peaks of IFN- $\gamma$ , TNF, and IL-6 were observed in the bloodstreams and spleens of the mice with nonlethal and lethal infections within 24 h. Thereafter, in the nonlethal cases, IFN- $\gamma$ , TNF, and IL-6 peaked again in the spleens and kidneys during the period of maximum growth of bacteria in the kidneys, although only IL-6 was detected in the sera. In contrast, in the case of lethal infection, the titers of IFN- $\gamma$  and IL-6 in the sera and TNF in the kidneys peaked before death. Effects of in vivo administration of monoclonal antibodies (MAbs) against IFN- $\gamma$  and TNF on the fates of *S. aureus*-infected mice were studied. In the nonlethal infections, anti-TNF alpha (anti-TNF- $\alpha$ ) MAb-treated mice, but not anti-IFN- $\gamma$  MAb-treated mice, died as a result of worsening infection, suggesting that endogenous TNF plays a protective role in host resistance to *S. aureus* infection. In the mice that received lethal doses, injection of anti-TNF- $\alpha$  MAb accelerated death. However, although injection of anti-IFN- $\gamma$  MAb inhibited host resistance of the infected mice early in infection, most of the animals survived the lethal infection by injection of anti-IFN- $\gamma$  MAb, suggesting that endogenous IFN- $\gamma$  plays a detrimental role in *S. aureus* infection. Thus, this study demonstrated that IFN- $\gamma$  and TNF play different roles in *S. aureus* infection.**

Bacteria and their products induce various cytokines that play a significant role in host resistance. For example, gamma interferon (IFN- $\gamma$ ) and tumor necrosis factor (TNF), which are induced endogenously, play both beneficial and detrimental roles in hosts exposed to bacterial infections. These cytokines reportedly play a protective role in host resistance to facultative intracellular-growing bacteria, including *Listeria monocytogenes* (6, 19, 26, 35, 36, 44, 45), *Mycobacterium tuberculosis* (8, 16), *Mycobacterium bovis* BCG (9, 27), *Salmonella typhimurium* (42, 53), and *Francisella tularensis* (30). In contrast, IFN- $\gamma$  and TNF mediate gram-negative septic shock and endotoxin shock (3, 11, 20, 22, 47, 54).

Staphylococci, including *Staphylococcus aureus*, a gram-positive extracellular-growing bacterium, are a major source of morbidity and mortality in medical facilities. Recent clinical trials have shown an increase in the incidence of gram-positive bacterial sepsis despite improvements in supportive therapy (5, 51). Staphylococci and their products can induce various cytokines. Intact staphylococci and purified peptidoglycans have been shown to induce TNF, interleukin-1 (IL-1), and IFN- $\gamma$  in humans and animals in vitro and in vivo (23, 25, 52, 57). Furthermore, staphylococcal enterotoxins and toxic shock syndrome toxin 1, which are exotoxins produced by *S. aureus* and are members of the superantigen family, can induce cytokines such as TNF, IFN- $\gamma$ , IL-1, IL-2, and IL-6 (7, 13, 29, 33, 43). The antibodies against TNF reportedly protect animals from

lethal infections with *S. aureus* (17, 24) and staphylococcal enterotoxin B-induced shock (32), suggesting that TNF plays a detrimental role in *S. aureus* infection. However, another study (56) demonstrated that recombinant TNF alpha (TNF- $\alpha$ ) contributed to host defense against *S. aureus* infection. There are no reports on the role of IFN- $\gamma$  in *S. aureus* infection. To clarify the roles of these cytokines in *S. aureus* infection, we investigated the endogenous productions of IFN- $\gamma$ , TNF, and IL-6 induced by infections with lethal or nonlethal doses of *S. aureus* and the effects of in vivo administration of monoclonal antibodies (MAbs) against IFN- $\gamma$  and TNF during the course of infections in mice. In the present study, we demonstrate the protective role of TNF and the detrimental role of IFN- $\gamma$  in host responses to *S. aureus* infection.

### MATERIALS AND METHODS

**Mice.** Female ddY outbred mice (age, 5 weeks; obtained from SLC, Hamamatsu, Shizuoka, Japan) were used.

**Bacteria.** *S. aureus* 834 was a clinical isolate from a patient at Hokkaido University Hospital, Sapporo, Japan. The strain was classified as coagulase type II, produced staphylococcal enterotoxin C and toxic shock syndrome toxin 1, and was methicillin resistant. In each experiment, bacteria were cultured on nutrient agar (Nissui Pharmaceutical Co., Tokyo, Japan) for 24 h at 37°C, inoculated into tryptic soy broth (Difco Laboratories, Detroit, Mich.), and incubated for another 15 h. The organisms were collected by centrifugation and washed three times with 0.85% saline. The concentration of washed cells was adjusted spectrophotometrically at 550 nm. The numbers of viable *S. aureus* cells were established by plating serial 10-fold dilutions of a bacterial solution in 0.01 M phosphate-buffered saline (PBS; pH 7.4) on nutrient agar. Colonies were routinely counted 20 to 24 h later. Mice were infected intravenously with 0.2 ml of a solution containing 10<sup>7</sup> CFU (0.25 50% lethal dose) or 10<sup>8</sup> CFU (2.5 50% lethal dose) of viable *S. aureus* cells in PBS.

**In vivo depletion of endogenous cytokines.** Hybridoma cell lines secreting MAbs against mouse IFN- $\gamma$  (R4-6A2; rat immunoglobulin G1) (48), mouse

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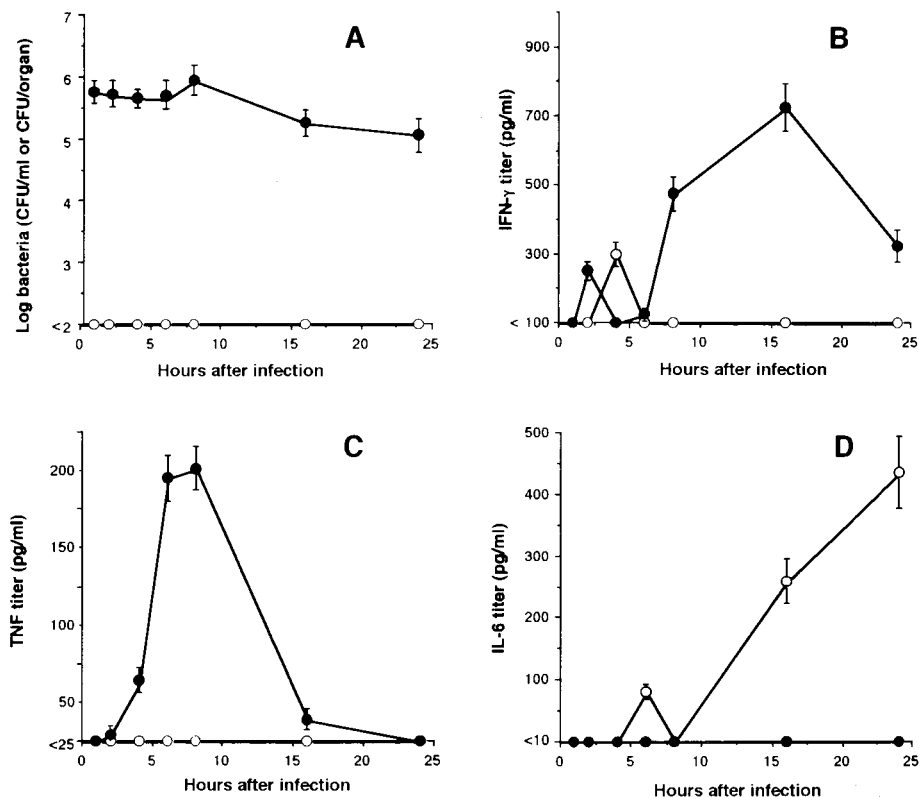


FIG. 1. Kinetics of endogenous cytokine production within 24 h after infection with a nonlethal dose of *S. aureus*. Mice were infected intravenously with  $10^7$  CFU of *S. aureus*. The numbers of viable *S. aureus* cells in the blood (○) and spleens (●) were determined (A). At the indicated times, in parallel, the titers of IFN- $\gamma$  (B), TNF (C), and IL-6 (D) in the sera (○) and spleens (●) were determined. Each point represents the mean  $\pm$  standard deviation for a group of five mice.

TNF- $\alpha$  (MP6-XT22.11; rat immunoglobulin G1) (1), and mouse IL-6 (MP5-20F3.11; rat immunoglobulin G1) (1) were used. MP6-XT22.11 and MP5-20F3.11 cells were kindly provided by J. S. Abrams, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, Calif. MAbs found in the ascites fluid were partially purified by 50%  $(\text{NH}_4)_2\text{SO}_4$  precipitation. The mice were given a single intravenous injection of each MAb 2 h before infection. Normal rat globulin (NRG) was injected as a control for the MAbs. NRG was prepared as described previously (38).

**Tests for endotoxin contamination.** All of the in vivo effects of MAbs and NRG described were verified to contain  $<0.1$  ng per injected dose by use of reagents tested by the *Limulus* amoebocyte lysate assay.

**Preparation of organ extracts.** The spleens or kidneys were suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 1% (wt/vol) 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfate (CHAPS; Wako Pure Chemicals Co., Osaka, Japan), and 10% (wt/vol) homogenates were prepared with a Dounce grinder and then clarified by centrifuging at  $2,000 \times g$  for 20 min (39). The organ extracts were stored at  $-70^\circ\text{C}$  until cytokine assays were performed.

**IFN- $\gamma$  assay.** The IFN- $\gamma$  assay was carried out by a double-sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (37). Purified rat anti-mouse IFN- $\gamma$  MAb produced by hybridoma R4-6A2 and rabbit anti-recombinant mouse IFN- $\gamma$  serum (37) were used for the ELISA. All ELISAs were run with recombinant mouse IFN- $\gamma$  produced and purified by Genentech, Inc., San Francisco, Calif.

**TNF assay.** The TNF assay was carried out with a double-sandwich ELISA as described previously (39). Purified hamster anti-recombinant mouse TNF- $\alpha$  MAb (Genzyme Co., Boston, Mass.) and rabbit anti-recombinant mouse TNF- $\alpha$  globulin (35) were used for the ELISA. All ELISAs were run with recombinant mouse TNF- $\alpha$  (Genzyme).

**IL-6 assay.** The IL-6 content of test samples was assayed by monitoring their ability to cause proliferation of the IL-6-dependent mouse hybridoma cell line MH60.BSF-2 (34), as described previously (39). Each assay was calibrated in terms of the biological activity of an IL-6 laboratory reference standard (purified recombinant human IL-6 provided by T. Matsuda and T. Hirano of Biomedical Research Center, Osaka University Medical School, Osaka, Japan).

**Statistical evaluation of the data.** Data were expressed as means  $\pm$  standard deviations, and the Wilcoxon rank sum test was used to determine the significance of the differences of bacterial counts in the organs or the cytokine titers

between the control and experimental groups. The generalized Wilcoxon test was used to determine the significance of differences in the survival rate. Each experiment was repeated at least twice and accepted as valid only when the trials showed similar results.

## RESULTS

**Kinetics of bacterial growth and endogenous IFN- $\gamma$ , TNF, and IL-6 production within 24 h in mice which were infected with a nonlethal dose of *S. aureus*.** Mice were infected intravenously with 0.25 50% lethal dose of viable *S. aureus*, and the bacterial growth in the bloodstreams and spleens was determined from 1 to 24 h after infection (Fig. 1A). The *S. aureus* cells injected were eliminated rapidly from the bloodstreams, and they were already below a detectable level at 1 h, while a large number of bacteria was detected in the spleens and maintained until 8 h, thereafter decreasing gradually. In parallel, the titers of IFN- $\gamma$ , TNF, and IL-6 were determined in the sera and spleen homogenates (Fig. 1B to D). A marginal amount of IFN- $\gamma$  was detected at 4 h in the sera only, while IFN- $\gamma$  appeared in the spleens transiently at 2 h, reappeared at 8 h, and peaked at 16 h. In the spleens, TNF appeared at 4 h, peaked at 8 h, and then decreased. No TNF was detected in the sera. IL-6 increased at 16 and 24 h in the sera but was never detected in the spleens.

**Kinetics of bacterial growth and endogenous IFN- $\gamma$ , TNF, and IL-6 production after 24 h in mice which were infected with a nonlethal dose of *S. aureus*.** The presence of *S. aureus* cells in the blood, spleens, and kidneys of the mice was observed up to 4 weeks after infection (Fig. 2A). A marginal number of *S. aureus* cells was detected in the blood only up to

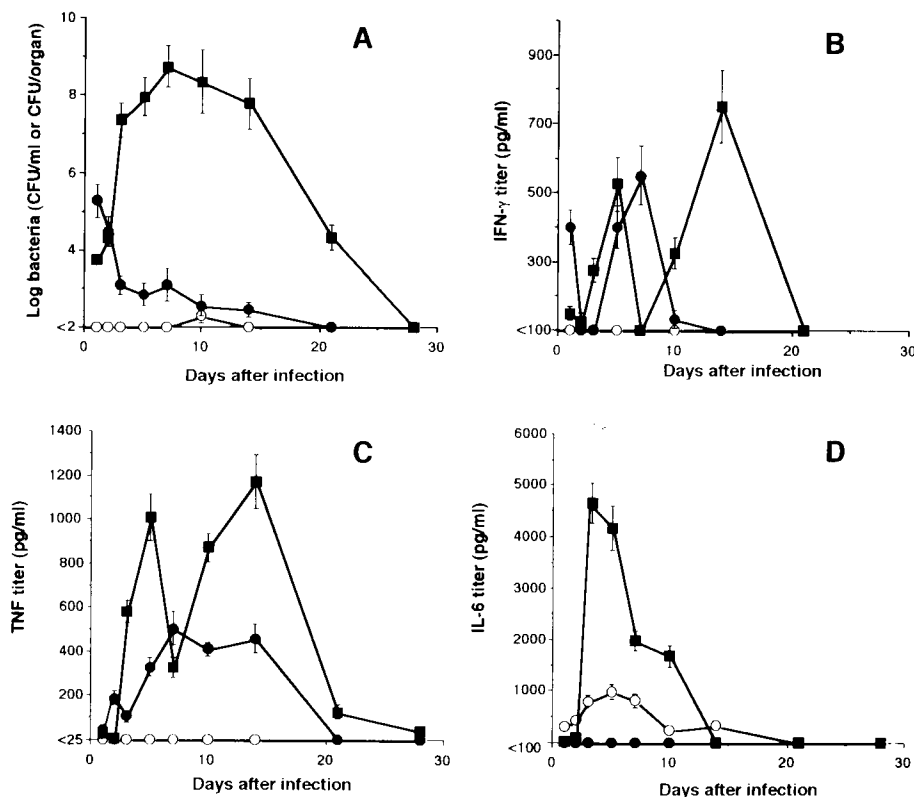


FIG. 2. Kinetics of *S. aureus* infection and endogenous cytokine production after 24 h of infection with a nonlethal dose of *S. aureus*. Mice were infected intravenously with  $10^7$  CFU of *S. aureus*, and the numbers of *S. aureus* cells in the blood ( $\circ$ ), spleens ( $\bullet$ ), and kidneys ( $\blacksquare$ ) were determined (A). In parallel, the titers of IFN- $\gamma$  (B), TNF (C), and IL-6 (D) in the sera ( $\circ$ ), spleens ( $\bullet$ ), and kidneys ( $\blacksquare$ ) were determined. Each point represents the mean  $\pm$  standard deviation for a group of five mice.

2 weeks after infection. The bacteria in the spleens decreased after 1 day but could be detected up to 3 weeks postinfection. In contrast, the numbers of *S. aureus* cells in the kidneys increased from day 1 and peaked on day 7. Constant bacterial numbers were observed until 2 weeks, after which they decreased and finally disappeared 4 weeks postinfection. The IFN- $\gamma$  titers in the sera, spleen homogenates, and kidney homogenates of the *S. aureus*-infected mice were determined from 1 to 28 days after infection (Fig. 2B). No IFN- $\gamma$  was detected in the serum specimens. Endogenous IFN- $\gamma$  production in the spleens peaked on day 7 of infection. The IFN- $\gamma$  titers in the kidneys showed a bimodal curve, namely, they peaked on days 5 and 14. Although no TNF was detected in any serum specimens, it was observed in the spleens and kidneys until *S. aureus* cells disappeared from these organs. The TNF titers in the spleens peaked from day 7 to day 14 of infection, and then the cytokine disappeared (Fig. 2C). In the kidneys, TNF increased rapidly on day 3, and the titers showed a bimodal curve, namely, they peaked on days 5 and 14. While in the sera, IL-6 was detected until 14 days and peaked on day 5 of infection (Fig. 2D). No spleen specimens showed IL-6 activity. High IL-6 titers were observed in the kidneys on day 3 of infection, but they declined and disappeared before the *S. aureus* cells were eliminated.

**Effect of in vivo administration of MABs against IFN- $\gamma$  and TNF on the survival rates of mice which were infected with a nonlethal dose of *S. aureus*.** One milligram each of anti-IFN- $\gamma$  MAB, anti-TNF- $\alpha$  MAB, or NRG was injected intravenously into mice 2 h before *S. aureus* infection, and the survival of each group was observed for 21 days (Fig. 3). All of the anti-

IFN- $\gamma$  MAB-treated mice as well as the control mice which received NRG survived, whereas 64% of the anti-TNF- $\alpha$  MAB-treated mice died within 10 days of infection.

**Effect of in vivo administration of MABs against IFN- $\gamma$  and TNF on the growth of *S. aureus* cells in the bloodstreams and organs of mice infected with a nonlethal dose of *S. aureus*.**

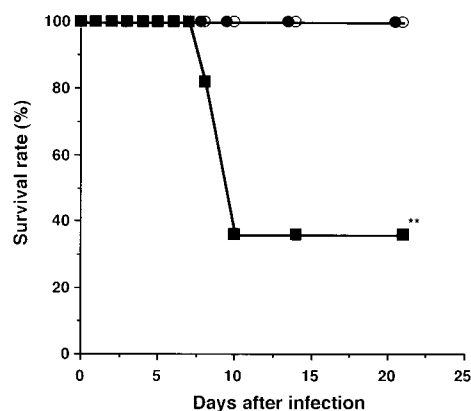


FIG. 3. Effect of anti-cytokine MABs on survival rates of mice infected with a nonlethal dose of *S. aureus*. Each of 22 mice per group was infected intravenously with 1 mg of NRG ( $\circ$ ), anti-IFN- $\gamma$  MAB ( $\bullet$ ), or anti-TNF- $\alpha$  MAB ( $\blacksquare$ ) 2 h before infection with  $10^7$  CFU of *S. aureus*. A double asterisk indicates a significant difference for the value for the NRG-treated group at  $P$  of  $<0.01$ .

TABLE 1. Effect of in vivo administration of MAbs against IFN- $\gamma$  and TNF- $\alpha$  on growth of *S. aureus* in the bloodstreams and organs of mice which were infected with the nonlethal dose of *S. aureus*

Mice treated with <sup>a</sup> :	Sampling day	No. of <i>S. aureus</i> cells <sup>b</sup> in:		
		Blood (log CFU/ml)	Spleen (log CFU/organ)	Kidney (log CFU/organ)
NRG	2	<2	4.55 $\pm$ 0.23	4.70 $\pm$ 0.21
Anti-IFN- $\gamma$ MAb	2	2.30 $\pm$ 0.14	4.23 $\pm$ 0.18	5.00 $\pm$ 0.28
Anti-TNF- $\alpha$ MAb	2	3.48 $\pm$ 0.28	4.59 $\pm$ 0.25	5.90 $\pm$ 0.26 <sup>c</sup>
NRG	8	<2	2.26 $\pm$ 0.14	6.45 $\pm$ 0.43
Anti-IFN- $\gamma$ MAb	8	<2	2.26 $\pm$ 0.22	4.67 $\pm$ 0.25 <sup>c</sup>
Anti-TNF- $\alpha$ MAb	8	3.32 $\pm$ 0.24	3.85 $\pm$ 0.23 <sup>c</sup>	9.21 $\pm$ 0.75 <sup>c</sup>

<sup>a</sup> Each globulin (1 mg per mouse) was injected intravenously 2 h before infection.

<sup>b</sup> Each result represents the mean  $\pm$  standard deviation for a group of five mice.

<sup>c</sup> Significantly different from value for NRG-treated group for same day ( $P < 0.01$ ).

Mice were injected intravenously with NRG, anti-IFN- $\gamma$  MAb, or anti-TNF- $\alpha$  MAb. The numbers of viable *S. aureus* cells in the bloodstreams, spleens, and kidneys were determined 2 and 8 days later. On day 2 of infection (Table 1), no *S. aureus* cells were detected in the blood of the NRG-treated mice, but the bacteria were detected in the blood of the anti-IFN- $\gamma$  MAb-treated and anti-TNF- $\alpha$  MAb-treated mice. The number of *S. aureus* cells in the blood of mice which received anti-TNF- $\alpha$  MAb was significantly higher than that of the anti-IFN- $\gamma$  MAb-treated mice ( $P < 0.01$ ). The numbers of *S. aureus* cells in the spleens were comparable among the three groups. In the kidneys, the bacterial numbers in the anti-TNF- $\alpha$  MAb-treated mice were significantly higher than those of NRG-treated or anti-IFN- $\gamma$  MAb-treated mice ( $P < 0.01$ ). On day 8 of infection (Table 1), the administration of anti-TNF- $\alpha$  MAb resulted in a significant increase in the bacterial numbers in both specimens ( $P < 0.01$ ). However, in the kidneys, the growth of *S. aureus* was inhibited in the anti-IFN- $\gamma$  MAb-treated mice ( $P < 0.01$ ).

**Kinetics of bacterial growth and endogenous IFN- $\gamma$ , TNF, and IL-6 production within 24 h in mice infected with a lethal dose of *S. aureus*.** When mice were infected intravenously with  $10^8$  CFU of *S. aureus* cells, which is equivalent to 2.5 50% lethal doses, all of them died within 7 days postinfection (Fig. 4, NRG-treated group). Bacterial growth in the bloodstreams and spleens was determined from 1 to 24 h after infection (Fig. 5A). The *S. aureus* cells injected were eliminated rapidly from the bloodstreams, and they were already under the detectable level at 1 h, while the bacteria gradually increased 6 h later. In parallel, the titers of IFN- $\gamma$ , TNF, and IL-6 were determined in the sera and spleen homogenates (Fig. 5B to D). IFN- $\gamma$  was detected at 4 h and peaked at 6 h, and the higher titers of IFN- $\gamma$  were detected at 16 and 24 h in the bloodstreams, while IFN- $\gamma$  production in the spleens peaked at 8 h. TNF appeared in the spleens at 1 h, peaked at 8 h, and then decreased. Although a marginal amount of TNF was detected in the sera at 1 h, no TNF was detected thereafter. In both the sera and spleens, IL-6 peaked at 6 h and then decreased.

**Kinetics of bacterial growth and endogenous IFN- $\gamma$ , TNF, and IL-6 production after 24 h in mice infected with a lethal dose of *S. aureus*.** *S. aureus* cells and the titers of endogenous cytokines were determined in the bloodstreams, spleens, and kidneys until day 5 of infection (Fig. 6). In the blood, *S. aureus*

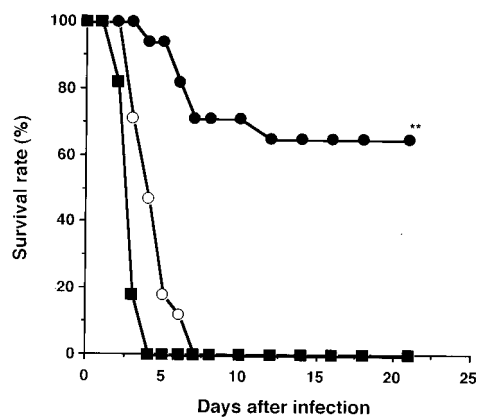


FIG. 4. Effect of anti-cytokine MAbs on survival rates of mice infected with a lethal dose of *S. aureus*. Each of 17 mice per group was injected intravenously with 1 mg of NRG ( $\circ$ ), anti-IFN- $\gamma$  MAb ( $\bullet$ ), or anti-TNF- $\alpha$  MAb ( $\blacksquare$ ) 2 h before infection with  $10^8$  CFU of *S. aureus*. A double asterisk indicates a significant difference for the value for the NRG-treated group at  $P < 0.01$ .

cells were detected persistently until death (Fig. 6A). Although the numbers of bacteria in the spleens decreased slightly for 3 days, they increased before death. The numbers of *S. aureus* cells in the kidneys increased until death. In parallel, the titers of endogenous IFN- $\gamma$ , TNF, and IL-6 were observed in the sera and spleen and kidney homogenates of *S. aureus*-infected mice (Fig. 6B to D). In the bloodstreams, IFN- $\gamma$  titers decreased after 1 day, and the high titers of IFN- $\gamma$  appeared before death. IFN- $\gamma$  titers in the spleens decreased after 1 day and barely increased before death. Titers of TNF in the spleens peaked on day 2 of infection and reached an undetectable level before death, while TNF in the kidneys increased markedly, depending on the period of infection. The kinetics of IL-6 titers in the bloodstreams were similar to those of the TNF titers in the kidneys, while constant IL-6 levels were detected in the spleens and kidneys for up to 5 days.

**Effect of in vivo administration of MAbs against IFN- $\gamma$  and TNF on the survival rates of mice infected with a lethal dose of *S. aureus*.** One milligram of anti-IFN- $\gamma$  MAb, anti-TNF- $\alpha$  MAb, or NRG was injected intravenously into mice 2 h before the lethal infection with *S. aureus*, and the survival of each group was observed for 21 days (Fig. 4). All of the NRG-treated mice died within 7 days. The anti-TNF- $\alpha$  MAb-treated mice died rapidly, while the anti-IFN- $\gamma$  MAb-treated mice survived for a significantly longer period of time than the NRG-treated animals ( $P < 0.01$ ).

**Effect of in vivo administration of MAbs against IFN- $\gamma$  and TNF- $\alpha$  on bacterial growth in the bloodstreams and organs of mice infected with a lethal dose of *S. aureus*.** Mice were injected with 1 mg of anti-IFN- $\gamma$  MAb, anti-TNF- $\alpha$  MAb, or NRG 2 h before the lethal *S. aureus* infection, and the numbers of bacterial cells in the blood, spleens, and kidneys were determined on days 1 and 4 of infection (Table 2). On day 1, the numbers of *S. aureus* cells in the blood, spleens, and kidneys of both anti-IFN- $\gamma$  MAb-treated and anti-TNF- $\alpha$  MAb-treated mice were higher than those of NRG-treated mice. In contrast, on day 4, the bacterial numbers in the blood and kidneys of NRG-treated mice increased, while *S. aureus* cells decreased markedly in the anti-IFN- $\gamma$  MAb-treated mice. The bacterial numbers in the anti-TNF- $\alpha$  MAb-treated mice could not be determined because most of the mice died within 4 days.



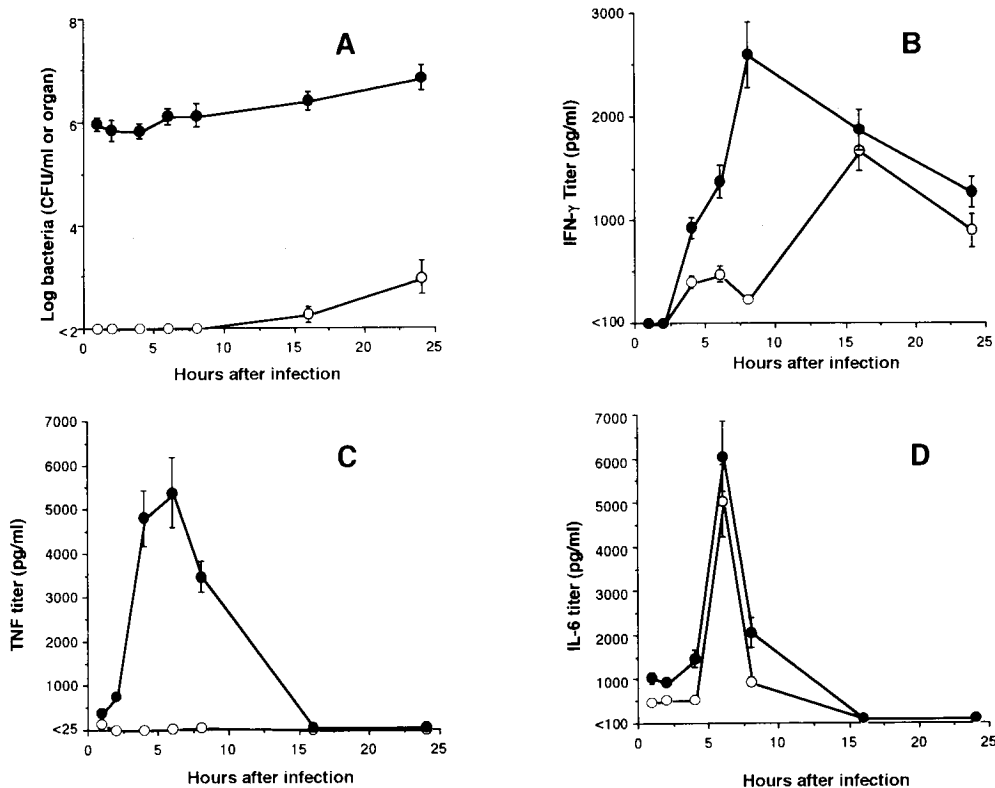


FIG. 5. Kinetics of endogenous cytokine production within 24 h after infection with a lethal dose of *S. aureus*. Mice were infected intravenously with  $10^8$  CFU of *S. aureus*. The numbers of viable *S. aureus* cells in the blood ( $\circ$ ) and spleens ( $\bullet$ ) were determined (A). At the indicated time, in parallel, the titers of IFN- $\gamma$  (B), TNF (C), and IL-6 (D) in the sera ( $\circ$ ) and spleens ( $\bullet$ ) were determined. Each point represents the mean  $\pm$  standard deviation for a group of five mice.

## DISCUSSION

The present study demonstrated that endogenous TNF plays a protective role in host resistance to *S. aureus* infection, but endogenous IFN- $\gamma$  provides protection in the early stage of infection and plays a detrimental role late in infection.

In addition to skin infections, *S. aureus* often causes life-threatening, deep-seated infections, including abscesses of various organs, osteomyelitis, pyelonephritis, pneumonia, meningitis, purulent arthritis, endocarditis, and sepsis. In our preliminary experiments, in which mice were injected intravenously with either nonlethal or lethal doses, significant proliferations of *S. aureus* cells along with abscess formation were observed in the kidneys, whereas bacterial numbers were fewer in other organs, including the spleens, livers, and lungs. Therefore, we focused on the kidneys, spleen, and blood to evaluate the proliferation of *S. aureus* cells and cytokine production.

The beneficial and detrimental roles of TNF in bacterial infections have been reported. TNF plays a central role in host resistance to various facultative intracellular-growing bacteria (19, 30, 35, 36, 42, 44, 45, 53). In contrast, TNF has been identified as an essential mediator in septic shock induced by gram-negative bacteria (3, 54). In *S. aureus* infections, infusion of bacterial cells into dogs, rabbits, or primates reportedly resulted in the induction of TNF and caused cardiovascular dysfunction similar to that seen in human sepsis (25, 40). The role of TNF was demonstrated in studies of mice and primates (17, 24) in which antibodies against TNF- $\alpha$  protected animals from the lethal effects of *S. aureus*. Furthermore, anti-TNF- $\alpha$  antibodies protected mice from the lethal effects of staphylococcal enterotoxin B (32). In the present study, however, *S.*

*aureus* infection worsened by in vivo administration of anti-TNF- $\alpha$  MAb (Fig. 3). *S. aureus* was detected in the bloodstreams and significantly higher numbers of bacteria were observed in the kidneys of anti-TNF- $\alpha$  MAb-treated mice which received the nonlethal infection (Table 1). Furthermore, in vivo administration of anti-TNF- $\alpha$  MAb accelerated a decrease in the survival time of mice which received the lethal infection (Fig. 4). These results suggest that endogenous TNF plays an important role in host resistance to *S. aureus* infection. The contribution of TNF to host resistance to *S. aureus* infection was also demonstrated in guinea pigs injected with recombinant mouse TNF- $\alpha$ , which showed a prophylactic effect on the infection (56). The present results are inconsistent with other reports showing that endogenous TNF plays a detrimental role in *S. aureus* infection (17, 24, 25, 32). The differences may be explained as follows. First, the previous studies were carried out with live or killed bacteria or staphylococcal enterotoxin B, which caused death in animals within 24 h, whereas we used nonlethal or lethal doses of *S. aureus*, which caused death in mice after 3 days. Second, TNF was detected in the bloodstreams of the animals in the other studies, whereas TNF was barely detectable in the sera of mice which received either the nonlethal or lethal infections in the present study (Fig. 1, 2, 5, and 6). In the present study, endogenous cytokine production in either nonlethal or lethal infections with *S. aureus* could be divided into two phases. IFN- $\gamma$ , TNF, and IL-6 production was induced initially within several hours after infection and decreased thereafter (Fig. 1 and 5). Later, endogenous cytokine production increased in parallel with bacterial growth in the kidneys (Fig. 2 and 6). Therefore, it is likely that the high TNF

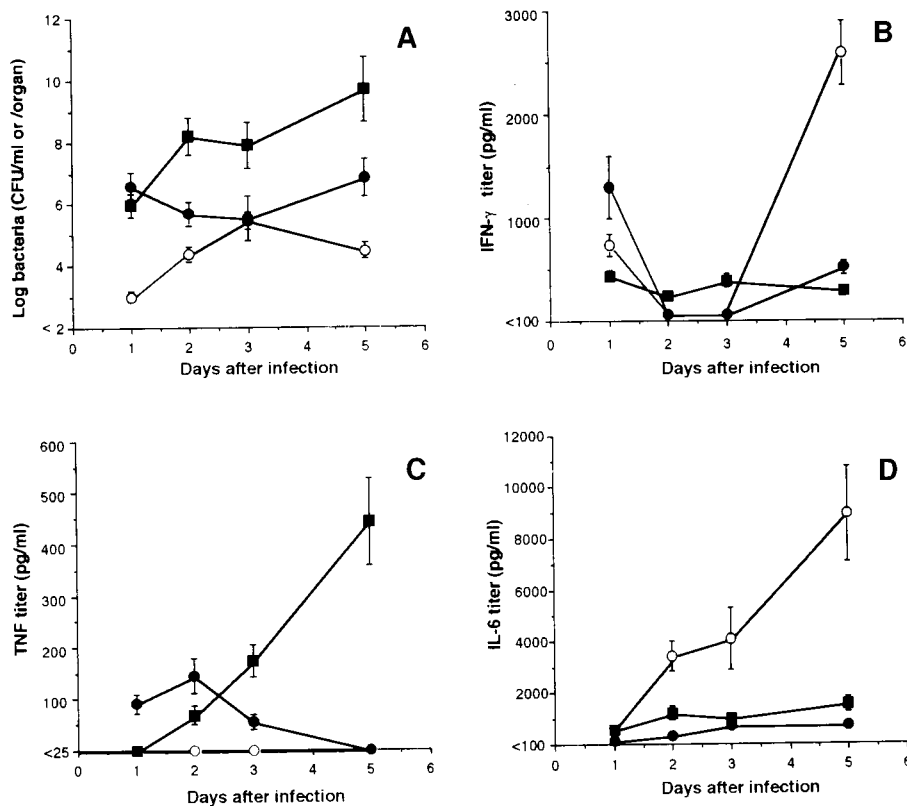


FIG. 6. Kinetics of *S. aureus* infection and endogenous cytokine production after 24 h of infection with a lethal dose of *S. aureus*. Mice were infected intravenously with  $10^8$  CFU of *S. aureus*, and the numbers of *S. aureus* cells in the blood (○), spleens (●), and kidneys (■) were determined (A). In parallel, the titers of IFN- $\gamma$  (B), TNF (C), and IL-6 (D) in the sera (○), spleens (●), and kidneys (■) were determined. Each point represents the mean  $\pm$  standard deviation for a group of five mice. The survivors were used for the analyses on days 3 and 5.

titers that were released rapidly into the circulation in the first phase by the administration of high doses of *S. aureus* cells or their exotoxins demonstrated a detrimental effect on the hosts, whereas the moderate titers of TNF induced in the infected foci by the proliferated *S. aureus* cells and their secreted exo-

toxins in either the first or the second phase might play the protective role in the host resistance.

Neutrophils are known to play an important role in host resistance to *S. aureus* infection (10). TNF has been shown to alter many properties of neutrophils in vitro: enhancement of phagocytosis, induction of degranulation, stimulation of adherence, and stimulation of the generation of superoxides (14, 18, 28, 41, 46, 49, 55). Furthermore, TNF enhances neutrophil complement-dependent killing of *S. aureus*, and the effect involves the increase of complement receptor 3 (CD11b/CD18) and complement receptor 4 (P150, 95; CD11c/CD18) (15). Additionally, TNF induces the expression of E-selectin (4) on endothelial surfaces and is a chemotactic factor (2). In the present study, in vivo administration of anti-TNF- $\alpha$  MAb resulted in enhanced growth of *S. aureus* cells in the kidneys and dissemination of bacteria into the circulation of the infected mice either early in infection or before death (Tables 1 and 2). Therefore, the inhibition of enhancement of neutrophil functions, including bactericidal activity, might be involved in the effect of anti-TNF- $\alpha$  MAb.

Endogenous IL-6 was induced in the bloodstreams, spleens, and kidneys of *S. aureus*-infected mice (Fig. 1, 2, 5, and 6). We investigated the effect of in vivo administration of anti-IL-6 MAb on *S. aureus* infection (data not shown). The number of *S. aureus* cells increased in the kidneys of anti-IL-6 MAb-treated mice which received the nonlethal dose of the bacterium, although the effect was less than that of anti-TNF- $\alpha$  MAb. Recent studies showed that anti-IL-6 MAb, which formed an antigen-antibody complex, stabilized biologically

TABLE 2. Effect of in vivo administration of MAbs against IFN- $\gamma$  and TNF- $\alpha$  on growth of *S. aureus* in the bloodstreams and organs of mice which were infected with the lethal dose of *S. aureus*

Mice treated with <sup>a</sup> :	Sampling day	No. of <i>S. aureus</i> cells <sup>b</sup> in:		
		Blood (log CFU/ml)	Spleen (log CFU/organ)	Kidney (log CFU/organ)
NRG	1	3.85 $\pm$ 0.31	6.38 $\pm$ 0.54	7.21 $\pm$ 0.26
Anti-IFN- $\gamma$ MAb	1	4.57 $\pm$ 0.15 <sup>c</sup>	7.18 $\pm$ 0.35 <sup>d</sup>	7.92 $\pm$ 0.41 <sup>d</sup>
Anti-TNF- $\alpha$ MAb	1	4.71 $\pm$ 0.18 <sup>c</sup>	7.30 $\pm$ 0.43 <sup>d</sup>	8.35 $\pm$ 0.28 <sup>c</sup>
NRG	4	4.70 $\pm$ 0.48	6.04 $\pm$ 0.38	9.80 $\pm$ 0.70
Anti-IFN- $\gamma$ MAb	4	2.96 $\pm$ 0.35 <sup>c</sup>	3.31 $\pm$ 0.28 <sup>c</sup>	7.54 $\pm$ 0.48 <sup>c</sup>
Anti-TNF- $\alpha$ MAb	4	ND <sup>e</sup>	ND <sup>e</sup>	ND <sup>e</sup>

<sup>a</sup> Each globulin (1 mg per mouse) was injected intravenously 2 h before infection.

<sup>b</sup> Each result represents the mean  $\pm$  standard deviation for a group of five mice.

<sup>c</sup> Significantly different from value for NRG-treated group for same day ( $P < 0.01$ ).

<sup>d</sup> Significantly different from value for NRG-treated group for same day ( $P < 0.05$ ).

<sup>e</sup> ND, not determined. All mice died before day 4 of infection.

active IL-6, and the complex induced IL-6 (21, 31). Therefore, evaluation of the role of IL-6 seems to be difficult at present.

IFN- $\gamma$  reportedly plays a protective role in host resistance to facultative intracellular-growing bacteria (6, 8, 9, 16, 26, 27, 30, 36, 42). The synergistic effect of IFN- $\gamma$  and TNF on host resistance to *L. monocytogenes* infection has been reported (36). In the present study, the numbers of *S. aureus* cells in the bloodstreams, spleens, and kidneys of anti-IFN- $\gamma$  MAb-treated mice increased, compared with those in the control group, on day 1 of the lethal infection (Table 2), suggesting that IFN- $\gamma$  as well as TNF might play a protective role early in *S. aureus* infection. Surprisingly, however, the bacterial numbers in the specimens of anti-IFN- $\gamma$  MAb-treated mice decreased, compared with those in the control group, just before the control mice died (Table 2). Furthermore, in vivo administration of anti-IFN- $\gamma$  MAb protected mice from the lethal infection (Fig. 4). Inhibition of bacterial growth by the administration of anti-IFN- $\gamma$  MAb was also observed in the kidneys of the mice on day 8 of the nonlethal infection (Table 1). These results suggested that endogenous IFN- $\gamma$  plays a detrimental role in *S. aureus* infection. Recently, several reports showed that IFN- $\gamma$  plays a central role in septic shock induced by gram-negative bacteria (11, 20, 22, 47). The synergistic toxicity of TNF- $\alpha$  and IFN- $\gamma$  or negative control of TNF receptors of macrophages by IFN- $\gamma$  has been reported (12, 50). However, it is unlikely that anti-IFN- $\gamma$  MAb inhibits TNF-mediated toxicity because the administration of anti-TNF- $\alpha$  MAb failed to protect mice from lethal *S. aureus* infection (Fig. 4). Furthermore, excess TNF production in the bloodstreams was not observed in the anti-IFN- $\gamma$  MAb-treated mice (data not shown). More extensive studies are necessary to clarify the precise mechanism of IFN- $\gamma$  action in the pathology of *S. aureus* infection. Studies are planned to investigate the roles of T cells and superantigens in IFN- $\gamma$ -mediated lethality by *S. aureus* infection and to explore the possibility of immunotherapy for cases of severe *S. aureus* infection by regulation of endogenous IFN- $\gamma$ .

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