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Interactions between Endogenous Gamma Interferon and Tumor Necrosis Factor in Host Resistance against Primary and Secondary *Listeria monocytogenes* Infections

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Intravenous injection of rat anti-mouse gamma interferon (IFN-γ) monoclonal antibody as well as rabbit anti-mouse tumor necrosis factor (TNF) antibody into mice which had received a sublethal infection with *Listeria monocytogenes* cells resulted in acceleration of listeriosis. Endogenous IFN-γ seemed to be produced early in infection, because suppression of antilisterial resistance was significant when a single injection of anti-IFN-γ monoclonal antibody was given on day 0 or day 1 of infection. Production of TNF but not of IFN-γ in the bloodstream early in infection was inhibited by administration of anti-IFN-γ monoclonal antibody. The suppressive effect of anti-IFN-γ and anti-TNF antibodies on antilisterial resistance was not augmented by simultaneous administration of these antibodies. On the other hand, in the secondary infection, simultaneous administration of anti-IFN-γ and anti-TNF antibodies, but not of either of these antibodies alone, into *L. monocytogenes*-immune mice resulted in high mortality and explosive multiplication of bacterial cells in the spleens and livers. These results suggest that endogenously produced IFN-γ and TNF are both essential to the host defense against *L. monocytogenes* infection and that these cytokines might act by different modes between the primary infection and the secondary infection.

Complete elimination of *Listeria monocytogenes*, a facultative intracellular pathogen, from the tissues of infected animals is performed by macrophages activated by T-cell-dependent mechanisms (15, 20). Cytokines, produced mainly by immunocompetent cells, play crucial roles in establishment and expression of various immune responses. Production of endogenous cytokines involving gamma interferon (IFN-γ) (3), IFN-α (16), tumor necrosis factor (TNF) (9, 17), membrane-associated interleukin-1 (13), and colony-stimulating factors (5, 24) has been reported for *L. monocytogenes*-infected mice. Furthermore, the essential role of endogenously produced cytokines involving IFN-γ and TNF in antilisterial resistance has been demonstrated by in vivo injections of antibodies against these factors. Buchmeier and Schreiber (3) have reported that inhibition of clearance of *L. monocytogenes* cells and a higher mortality rate were found in mice treated with anti mouse-IFN-γ monoclonal antibody (MAb). Thereafter, Havell (9) demonstrated that TNF is another essential factor for antilisterial resistance by showing that injection of anti-mouse TNF-α antibody into sublethally infected mice resulted in increased bacterial growth in the organs and ultimately in death from listeriosis. Moreover, we showed that an undetectable level of endogenous TNF is produced early in *L. monocytogenes* infection and that suppression of antilisterial resistance by administration of anti-TNF antibody is due to inhibition of generation of activated macrophages rather than specific T cells (17).

The intimate interaction between TNF and IFN, especially IFN-γ, has been reported before. For example, TNF and IFN-γ have a synergistic effect on different biological activities involving enhancement of tumoricidal and schistosomulicidal activities and Ia expression of macrophages (4, 6). We focused our studies on in vivo analysis of the cooperative roles of endogenously produced IFN-γ and TNF in host defense mechanisms against primary and secondary infection with *L. monocytogenes* in mice in which these endogenous cytokines were depleted by administration of rat anti-mouse IFN-γ MAb and rabbit anti-mouse recombinant TNF-α antibody. In this report, we provide evidence that endogenous IFN-γ and TNF are both essential to the expression of antilisterial resistance against the primary infection and that the IFN-γ effect seems to be partially due to upregulation of TNF production. Contrary to this, our further results demonstrated that both IFN-γ and TNF are produced rapidly and might act without significant interaction in the secondary infection.

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MATERIALS AND METHODS

Mice. Female ddY mice (obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Shizuoka, Japan), 5 to 7 weeks old, were used.

Bacteria. *L. monocytogenes* 1b 1684 cells were prepared as described previously (18). The concentration of the washed cells was adjusted spectrophotometrically at 550 nm. Mice were infected intravenously with 0.2 ml of a solution containing 2×10^4 CFU of viable *L. monocytogenes* cells in 0.01 M phosphate-buffered saline (PBS; pH 7.4). In some experiments, they were reinfected with 5×10^6 CFU of viable bacterial cells 10 days later.

Anti-mouse IFN-γ antibody. Rat immunoglobulin G1 MAb against the purified preparation of mouse natural IFN-γ, which was prepared from the ascites fluid in Pristane-primed BALB/c nude mice injected with hybridoma R4-6A2 (8), was purified by DEAE Affi-Gel Blue column chromatography (2). Hybridoma R4-6A2 was donated by Y. Watanabe and Y.

Kawade, Institute for Virus Research, Kyoto University, Kyoto, Japan, with E. A. Havell's approval (Trudeau Institute, Inc., Saranac Lake, N.Y.). Purified MAb (1 mg) had a neutralizing titer of 2×10^5 against recombinant mouse IFN-γ (rMuIFN-γ). The characteristics of the MAb were described previously (21). To deplete endogenous IFN-γ in vivo, we gave each mouse a single intravenous injection of different concentrations of the immunoglobulin (0.2 ml) diluted with PBS 2 h before primary or secondary infections with *L. monocytogenes*. Purified normal rat globulin used as a control was purchased from Jackson Immunoresearch Laboratories, Inc., Avondale, Pa.

Anti-TNF-α antibody. An immunoglobulin fraction was prepared and purified by $(NH_4)_2SO_4$ precipitation from the serum of a rabbit hyperimmunized with purified recombinant mouse TNF-α (rMuTNF-α). The antibody (1 mg) specifically neutralized 2.4×10^5 U of the cytolytic activity of rMu-TNF-α, as described previously (17). To deplete endogenous TNF in vivo, we gave each mouse a single intravenous injection of the immunoglobulin fraction in the same manner as for the anti-IFN-γ MAb. Normal rabbit globulin used as a control was also prepared by $(NH_4)_2SO_4$ fractionation from the serum of a nonimmunized rabbit.

Tests for endotoxin contamination. All in vivo effects of antibodies or normal globulins described were verified by using reagents tested by the *Limulus* amoebocyte lysate assay to contain less than 0.1 ng per injected dose.

Induction of TNF. TNF was induced by injecting mice intravenously with 0.2 ml of a solution containing 25 μg of lipopolysaccharide (LPS) on day 1 of *L. monocytogenes* infection, and serum samples were obtained 1 h later. *Salmonella typhimurium* LPS, purified by the method of Westphal and Jann (23), was purchased from Difco Laboratories, Detroit, Mich.

Induction of IFN-α. After mice are infected intravenously with viable *L. monocytogenes* cells, IFN-α activity appears 24 h and reaches a maximum 48 h after infection (16). Therefore, IFN-α activity was determined by using the serum specimens obtained from mice 48 h after *L. monocytogenes* infection.

IFN assay. The IFN assay was carried out by the dye-binding method with mouse L-929 cells and vesicular stomatitis virus (Indiana strain) as previously described (16). The MAb neutralization assays of IFN-γ antiviral activities were carried out as previously described (19). The MAb neutralizing titer (neutralizing units [NU] per milliliter) is defined as the reciprocal of the dilution of antibody which, when reacted with an equal volume of rMuIFN-γ (20 IU/ml), neutralized 50% of the IFN activity. rMuIFN-γ was produced and purified by Genentech, Inc. (San Francisco, Calif.).

TNF assay. TNF titers were determined by the cytolytic activity against TNF-sensitive L-929 cells. Cells (2×10^4) suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with fetal calf serum (5%; GIBCO), penicillin G (100 U/ml), and streptomycin (100 μg/ml) were inoculated into 96-well flat-bottomed microplates (type 25860; Corning Glass Works, Corning, N.Y.), and the plates were incubated at 37°C in a 5% CO_2 incubator for 24 h. After the culture supernatant fluids were discarded, 0.1 ml of each sample diluted serially with the medium containing dactinomycin (2 μg/ml) was added to each well in duplicate. The cells were fixed and stained with 0.5% gentian violet–5% Formalin–50% ethanol in saline after further incubation at 37°C in a CO_2 incubator for 24 h. Titers are expressed as the reciprocal of the dilution of the sample in which 50% of the cells in the monolayer were lysed (17). rMuTNF-α was included in each assay as a laboratory standard. Neutralization assays of cytolytic activities with anti-mouse TNF antibody were carried out as previously described (17).

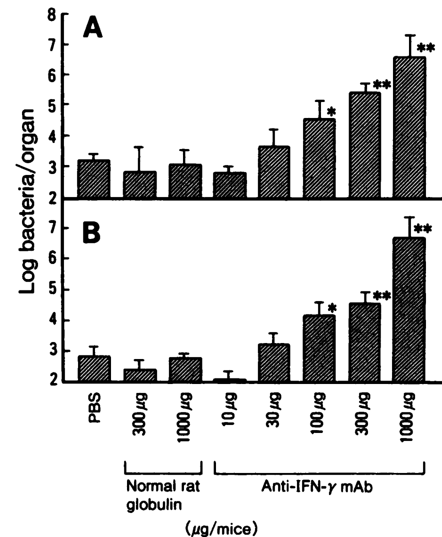

FIG. 1. Growth of *L. monocytogenes* cells late in infection in the spleens (A) and livers (B) of mice that received different doses of anti-IFN-γ MAb 2 h before infection. The number of bacteria in the organs was estimated 5 days later. Each result represents the mean ± standard deviation for a group of four mice. The results were reproduced in three different experiments. Symbols: * and **, significant difference from the value for PBS-treated mice at $P < 0.05$ and $P < 0.01$, respectively.

Determination of numbers of viable *L. monocytogenes* cells in the organs. The numbers of viable *L. monocytogenes* cells in the spleens and livers of the infected animals were established by plating serial 10-fold dilutions of organ homogenates in PBS on tryptic soy agar (Difco). Colonies were routinely counted 18 to 24 h later.

Statistical evaluation of the data. Data were expressed as mean ± standard deviation, and the Wilcoxon rank sum test was used to determine the significance of the differences of bacterial counts in the organs or of the titers of IFN and TNF between control and experimental groups. The *Z* test was used to determine the significance of differences in survival rate.

RESULTS

Effect of anti-IFN-γ MAb on antilisterial resistance against primary infection. Mice were injected intravenously with different doses of anti-mouse IFN-γ MAb or normal rat globulin (300 μg or 1 mg) 2 h before infection with a sublethal dose of the bacterium (2×10^4 CFU; 0.1 50% lethal dose). The survival of each group was observed for 14 days. Moreover, the number of *L. monocytogenes* cells in the spleens and livers of four mice in each group was determined on day 5 of infection, when antigen-specific elimination of bacteria had progressed (Fig. 1). No mouse died from infection even if 1 mg of the MAb had been injected (data not shown). However, elimination of bacteria from the spleens and livers was significantly blocked in mice that had received more than 100 μg of anti-IFN-γ MAb, depending on the doses of the MAb injected ($P < 0.05$ or $P < 0.01$).

Next, the effect of anti-IFN-γ MAb on nonspecific resistance early in *L. monocytogenes* infection was investigated.

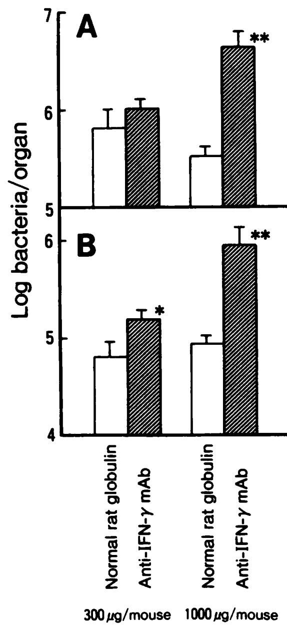

FIG. 2. Growth of *L. monocytogenes* cells early in infection in the spleens (A) and livers (B) of mice that received anti-IFN-γ MAb or normal rat globulin 2 h before infection. The number of bacteria in the organs was estimated 2 days later. Each result represents the mean ± standard deviation for a group of four mice. The results were reproduced in three different experiments. Symbols: * and **, significant difference from the value for normal rat globulin-treated mice at $P < 0.05$ and $P < 0.01$, respectively.

Mice were injected intravenously with 300 μg or 1 mg of anti-mouse IFN-γ MAb or normal rat globulin 2 h before infection, and the number of bacterial cells in the spleens and livers of these mice was determined on day 2 of infection (Fig. 2). The bacterial counts increased significantly in the livers of mice that had received 300 μg of anti-IFN-γ MAb ($P < 0.05$) and still more in both organs of the animals that had received 1 mg of the MAb ($P < 0.01$).

Effect of timing of administration of anti-IFN-γ MAb on antilisterial resistance. We assumed that endogenous IFN-γ would be produced and act on antilisterial resistance during the infection after administration of anti-IFN-γ MAb. A single injection of 1 mg of anti-IFN-γ MAb or normal rat globulin was given at different stages of infection, and the number of *L. monocytogenes* cells in the spleens and livers of the treated mice was determined on day 5 of infection (Table 1). Suppression of the elimination of bacteria from both organs was observed when mice received a single injection of the MAb on day 0 or 1 of infection (in the spleen, $P < 0.05$; in the liver, $P < 0.01$). In contrast, administration of anti-IFN-γ MAb on day 2 or 3 of infection had less effect on the elimination of bacteria from both organs ($P > 0.05$).

Effect of simultaneous administration of anti-IFN-γ and anti-TNF antibodies on resistance against primary listerial infection. Mice were injected intravenously with rabbit anti-mouse TNF-α immunoglobulin (50 μg), rat anti-mouse IFN-γ MAb (500 μg), or both 2 h before infection with a sublethal dose of *L. monocytogenes* cells. Normal rabbit globulin (50 μg), normal rat globulin (500 μg), or both were injected into mice as controls. The number of *L. monocytogenes* cells in the spleens and livers of the treated mice was determined on day 5 of infection (Fig. 3). Elimination of bacteria from the spleens and livers was significantly blocked in anti-IFN-γ MAb- and anti-TNF immunoglobulin-injected mice ($P < 0.01$). However, the suppressive effect of simultaneous administration of both antibodies against anti-

TABLE 1. Estimation of antilisterial resistance in mice after administration of anti-IFN-γ MAb on different days of infection

Time of injection (day postinfection)	Globulin injected[a]	Log no. of bacteria/organ[b]	
		Spleen	Liver
No injection	None	4.24 ± 0.32	3.35 ± 0.45
0 (−2 h)	Normal rat	4.07 ± 0.20	2.93 ± 0.33
	Anti-IFN-γ	5.43 ± 0.46[c]	4.49 ± 0.33[d]
1	Normal rat	4.20 ± 0.40	3.84 ± 0.32
	Anti-IFN-γ	5.29 ± 0.34[c]	5.82 ± 0.27[d]
2	Normal rat	4.88 ± 0.53	5.55 ± 0.55
	Anti-IFN-γ	4.65 ± 0.41	5.14 ± 0.32
3	Normal rat	4.87 ± 0.21	4.85 ± 0.43
	Anti-IFN-γ	4.65 ± 0.13	4.64 ± 0.40

[a] A single injection of normal rat globulin (1 mg) or anti-IFN-γ MAb (1 mg).
[b] The number of viable bacteria was determined on day 5 of infection. Each result represents the mean ± standard deviation for a group of four mice. The results were reproduced in three different experiments.
[c] Significantly different from value for normal rat globulin-treated group ($P < 0.05$).
[d] Significantly different from value for normal rat globulin-treated group ($P < 0.01$).

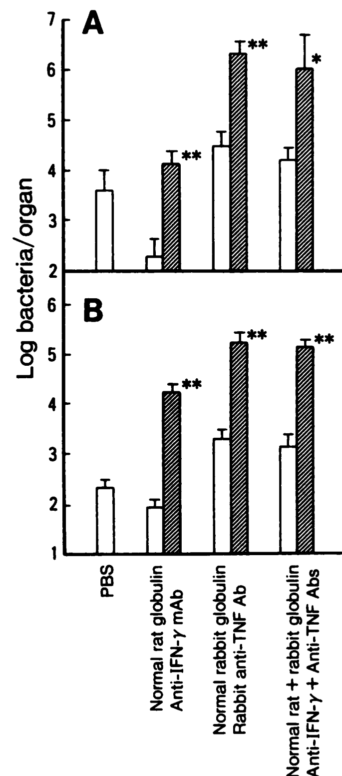

FIG. 3. Effect of simultaneous administration of anti-IFN-γ and anti-TNF antibodies on the growth of *L. monocytogenes* cells in the spleens (A) and livers (B) of mice in a primary infection. Antibodies, normal globulins, or PBS was injected 2 h before infection. The number of bacteria in the organs was estimated 5 days later. Each result represents the mean ± standard deviation for a group of four mice. The results were reproduced in three different experiments. Symbols: * and **, significant difference from the value for mice which received the corresponding normal globulin at $P < 0.05$ and $P < 0.01$, respectively. Ab, Antibody.

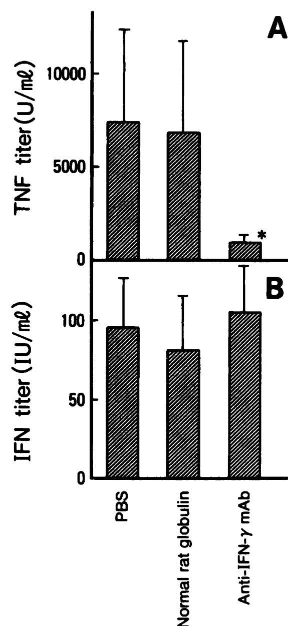

FIG. 4. Effect of in vivo administration of anti-IFN-γ MAb on production of TNF (A) and IFN-α (B) in the bloodstream of mice during *L. monocytogenes* infection. Anti-IFN-γ MAb, normal rat globulin, or PBS was injected 2 h before infection. TNF was induced by injection of LPS on day 1 of infection, and serum samples were taken 1 h later. Serum samples for the IFN-α assay were collected 48 h after infection without stimulation. Each result represents the mean ± standard deviation for a group of 15 mice from three experiments. Symbols: *, significant difference from the value for normal rat globulin-treated mice at $P < 0.01$.

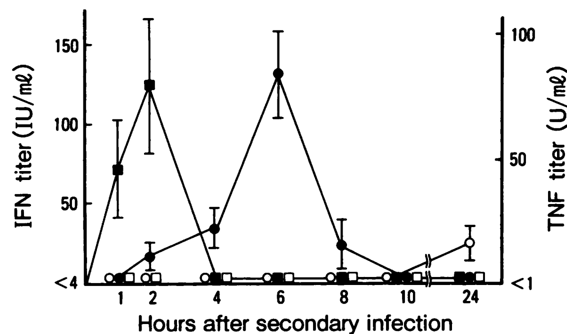

FIG. 5. Production of IFN and TNF in the bloodstream of mice following a secondary infection with *L. monocytogenes*. After 10 days of immunization, the immunized mice and nonimmunized controls were challenged with 5×10^6 CFU of viable *L. monocytogenes*, and blood samples were taken at different times postchallenge. The IFN activities of the sera from immunized mice (●) and nonimmunized mice (○) and the TNF activities of the sera from immunized mice (■) and nonimmunized mice (□) were determined. Each point represents the mean ± standard deviation for a group of 10 mice from two experiments.

listerial resistance was comparable to that after injection of either of the antibodies alone. For example, the number of bacteria in the spleens and livers between anti-TNF immunoglobulin-treated and both-antibody-treated mice was not significantly different ($P > 0.05$).

Effect of anti-IFN-γ MAb on TNF and IFN-α production in the bloodstream. We investigated the possibility that endogenously produced IFN-γ would regulate endogenous production of TNF during *L. monocytogenes* infection. Since our previous study (17) demonstrated that production of endogenous TNF in *L. monocytogenes*-infected mice occurred early in infection and the titer in serum was under the detectable level by a bioassay with L-929 cells, TNF production in vivo was amplified by stimulation of LPS (17). After mice which had been injected with anti-IFN-γ MAb (1 mg), normal rat globulin (1 mg), or PBS 2 h earlier were infected intravenously with *L. monocytogenes*, they were injected intravenously with LPS (25 μg) on day 1 of infection, and the serum samples for TNF titrations were taken 1 h later. TNF production in the bloodstream of *L. monocytogenes*-infected mice was significantly suppressed by treatment with anti-IFN-γ MAb (Fig. 4A). Similarly, we investigated the effect of anti-IFN-γ MAb on production of endogenous IFN-α, which is also produced early in infection (16). IFN-α activity in serum on day 2 of infection was determined (Fig. 4B). Administration of anti-IFN-γ MAb did not affect the production of endogenous IFN-α.

Production of IFN-γ and TNF in the bloodstream of mice during secondary infection. Mice were infected intravenously with 2×10^4 CFU of *L. monocytogenes* cells. After they were reinfected intravenously with 5×10^6 CFU of the bacterial cells on day 10 of infection, IFN and TNF activities in serum were monitored at various times (Fig. 5). IFN activity was demonstrated in the bloodstream 2 h after reinfection and peaked at 6 h. The IFN produced in the serum by reinfection was of the IFN-γ type (16). Alternatively, IFN production in the bloodstream of the nonimmunized controls started 24 h after infection, and the IFN produced was of the IFN-α type (16). On the other hand, TNF production in the bloodstream of the immunized mice was demonstrated 1 h after reinfection and peaked at 2 h. The TNF produced in the serum by reinfection was completely neutralized by rabbit anti-mouse TNF-α antibody (data not shown). No TNF was detected in the bloodstream of the nonimmunized controls.

Effect of anti-IFN-γ and anti-TNF antibodies on antilisterial resistance against secondary infection. Mice were immunized intravenously with 2×10^4 CFU of viable *L. monocytogenes* cells. On day 10 of immunization, they were injected intravenously with anti-IFN-γ MAb (500 μg), anti-TNF immunoglobulin (500 μg), or both 2 h before secondary challenge with 25 50% lethal doses (5×10^6 CFU) of *L. monocytogenes* cells. The survival of each group was observed for 10 days (Fig. 6). Every nonimmunized mouse died within 6 days of challenge, whereas the immunized controls which had received normal rat globulin (500 μg) and normal rabbit globulin (500 μg) simultaneously could escape from the lethal bacterial challenge. Likewise, neither anti-IFN-γ MAb- nor anti-TNF antibody-treated mice died. However, most of the animals which had received simultaneous administration of both antibodies could not escape from the lethal challenge even though they had been immunized.

To confirm that mice which had received both antibodies would develop severe listeriosis, the number of *L. monocytogenes* cells in the spleens and livers of four mice obtained from each group was determined on day 2 of the secondary challenge (Fig. 7). Administration of anti-IFN-γ MAb (500 μg) did not significantly affect the acquired antilisterial resistance ($P > 0.05$), and no effect was observed when even as much as 1 mg of the MAb was used (data not shown), while anti-TNF antibody significantly suppressed the elimination of bacteria from the organs ($P < 0.05$). In contrast, explosive multiplication of bacteria was observed in the

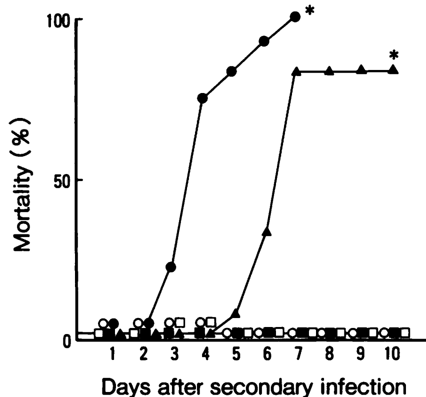

FIG. 6. Effect of simultaneous administration of anti-IFN-γ and anti-TNF antibodies on acquired resistance against a secondary infection with *L. monocytogenes*. The immunized mice received anti-IFN-γ MAb (□), anti-TNF antibody (■), or both (▲); the immunized controls received both normal rat and rabbit globulin (○) on day 10 of immunization. After 2 h, these mice and nonimmunized controls (●) were challenged with 25 50% lethal doses of viable *L. monocytogenes* cells. Each point represents a group of 12 mice from three experiments. Symbol: *, significant difference from the value for immunized mice which received both normal rat and rabbit globulin at $P < 0.001$.

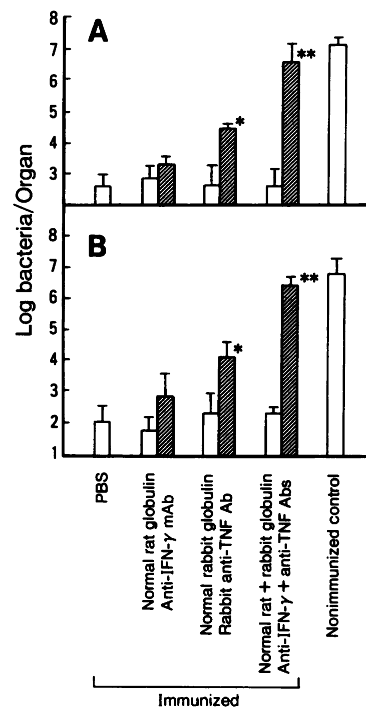

FIG. 7. Effect of simultaneous administration of anti-IFN-γ and anti-TNF antibodies on the growth of *L. monocytogenes* cells in the spleens (A) and livers (B) of mice after a secondary infection. The immunized mice received antibodies, normal globulins, or PBS on day 10 of immunization. After 2 h, these mice and nonimmunized controls were challenged with 25 50% lethal doses of viable *L. monocytogenes* cells. The number of bacteria in the organs was estimated 2 days later. Each result represents the mean ± standard deviation for a group of four mice. The results were reproduced in three different experiments. Symbols: * and **, significant difference from the value for mice which received the corresponding normal globulin at $P < 0.05$ and $P < 0.01$, respectively. Abs, Antibodies.

organs from mice which had received simultaneous administration of anti-IFN-γ and anti-TNF antibodies as well as in the nonimmunized controls ($P < 0.01$). The number of bacteria in both organs from mice which had received both antibodies was significantly higher than in organs from anti-IFN-γ MAb- or anti-TNF antibody-treated mice ($P < 0.01$).

DISCUSSION

The studies presented here demonstrated that endogenously produced IFN-γ and TNF both play essential roles in host defense against primary and secondary infections with *L. monocytogenes* and that the mode of interaction between IFN-γ and TNF in antilisterial resistance against primary infection is different from that in secondary infection.

We showed that administration of anti-IFN-γ MAb on day 1 of infection resulted in a decrease in antilisterial resistance, but no decrease occurred when the MAb was injected after day 1 (Table 1), suggesting that endogenous IFN-γ might be produced in the early phase of infection, although it has been reported that IFN-γ production induced by specific antigen occurs late in infection (3, 7, 10, 16). The source of IFN-γ early in infection has not been resolved. It is unlikely that sensitized T cells might produce IFN-γ in such an early phase. Bancroft et al. (1) demonstrated T-cell-independent production of endogenous IFN-γ in *L. monocytogenes*-infected mice with the severe combined immunodeficiency mutation. Our other study demonstrated that natural killer cells might be the principal source of IFN-γ in spleen cell cultures obtained from mice on day 2 of infection (manuscript in preparation). Experiments are in progress to test for the cellular source of IFN-γ in response to *L. monocytogenes* cells in vivo.

Although the depletion of endogenous IFN-γ resulted in partial inhibition of nonspecific resistance early in infection (Fig. 2), IFN-γ might act as an essential factor in acquired antilisterial resistance late in infection. Because injection of anti-IFN-γ MAb caused marked delay of elimination of bacteria from the organs late in infection (Fig. 1), endogenous IFN-γ may be produced too early to act on the acquired antilisterial resistance. However, Kurtz et al. (12) reported that administration of rMuIFN-γ concomitant with *L. monocytogenes* infection enhanced antilisterial resistance in the late phase but not in the early phase of infection, indicating that the IFN-γ effect shows a time lag. Moreover, our previous study also showed that administration of rMuIFN-γ 2 h before infection could restore the acquired antilisterial resistance late in infection in mice which had received an immunosuppressive treatment with cyclosporin A (19). Alternatively, van Dissel et al. (22) reported that rMuIFN-γ alone was not capable of activating the antibacterial activity of mouse peritoneal macrophages against *L. monocytogenes*. Therefore, endogenous IFN-γ may not activate antilisterial resistance directly but rather may modulate the system, e.g., through other cytokines involving TNF. Therefore, we investigated the effect of simultaneous administration of anti-IFN-γ and anti-TNF antibodies on antilisterial resistance. The inhibitory effect of both antibodies was comparable to that of either antibody alone (Fig. 3), suggesting that endogenous IFN-γ and TNF are both essential to antilisterial resistance and that either endogenous cytokine alone could not induce a significant host defense against a primary infection with *L. monocytogenes*. Furthermore, TNF production was suppressed in anti-IFN-γ MAb-treated

mice (Fig. 4), suggesting that endogenous IFN-γ might positively regulate TNF production in *L. monocytogenes*-infected mice. Our previous study (17) showed that endogenous TNF played an important role in generation of activated macrophages. Therefore, it is likely that IFN-γ would act on antilisterial resistance through TNF.

L. monocytogenes cells are rapidly eliminated from the organs of mice in a secondary infection, different from the case in a primary infection, even when a lethal dose of bacteria for nonimmunized animals was administered (14). This elimination seems to be the result of a quick and augmented response of immunocompetent cells involving T cells sensitized to listerial antigens (15, 20). Therefore, it is likely that the cellular responses of cytokine production would occur rapidly and intensively in a secondary infection. In fact, we were able to detect significant IFN-γ and TNF activities in the bloodstream of immunized mice soon after a secondary challenge with *L. monocytogenes* cells (Fig. 5). Significant quantities of TNF and IFN-γ had already appeared 1 and 2 h after secondary challenge, respectively.

The effects of anti-IFN-γ MAb and anti-TNF antibody on host resistance to a secondary infection with *L. monocytogenes* were different from the effects in a primary infection. We failed to block the elimination of bacteria from the organs of mice that received the secondary challenge by administration of anti-IFN-γ MAb (Fig. 7), although antiviral activities in the bloodstream of the MAb-treated mice were not detectable, different from the normal rat globulin-treated group (data not shown). Similarly, although mice received 500 μg of anti-TNF antibody in an experiment in which more than 50% of mice died from listeriosis in a primary infection (17), elimination of bacteria from the organs was significantly inhibited, but none of the mice died from the secondary challenge (Fig. 6 and 7). From these results, we cannot eliminate the possibility that the doses of these antibodies used may be insufficient to suppress the acquired antilisterial resistance or that substantial macrophage activation could still have been present after the primary infection. However, simultaneous administration of such doses of anti-IFN-γ and TNF antibodies into mice resulted in acceleration of death from a secondary infection with *L. monocytogenes* (Fig. 6). The treated mice died from listeriosis, because explosive multiplication of *L. monocytogenes* cells occurred in these mice as well as the nonimmunized controls (Fig. 7). The results suggest that both the anti-IFN-γ and anti-TNF antibodies administered suppress antilisterial resistance to a secondary infection synergistically. The results support the possibility that both IFN-γ and TNF produced endogenously might be essential to the full expression of antilisterial resistance to a secondary infection but that significant resistance might be able to develop even if only one of the cytokines is present.

On the basis of these observations, our present studies suggest that both IFN-γ and TNF are produced endogenously and are essential to antilisterial resistance to both primary and secondary infections. In addition to IFN-γ and TNF, other cytokines, including IFN-α, membrane-associated interleukin-1, and colony-stimulating factors, are reportedly produced endogenously during *L. monocytogenes* infection (5, 13, 16, 24). Numerous studies on the interactions and regulations between each cytokine have been reported. Therefore, it is necessary to consider the roles of endogenous IFN-α and TNF in host resistance to *L. monocytogenes* infection from the standpoint of interactions between these and other cytokines as well as between these two factors. We are now studying the roles of endogenous IFN-γ and TNF in antilisterial resistance to primary and secondary infections based on this concept.

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