INFECTION AND IMMUNITY, Oct. 1988, p. 2563–2569
0019-9567/88/102563-07$02.00/0

Vol. 56, No. 10

Endogenous Tumor Necrosis Factor (Cachectin) Is Essential to Host Resistance against *Listeria monocytogenes* Infection

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Received 15 March 1988/Accepted 21 June 1988

During a sublethal murine infection with *Listeria monocytogenes* cells, tumor necrosis factor (TNF) activity was detectable in neither sera nor spleen homogenates at any stage of the infection when a bioassay with L-929 cells (<4 U/ml) was used. However, injecting the mice with an immunoglobulin fraction obtained from a rabbit hyperimmunized with recombinant murine TNF-α resulted in acceleration of listeriosis. When 1 mg of anti-TNF antibody was injected per mouse, all the mice died from listeriosis, even though the infectious dose was sublethal for the untreated controls. The antigen-specific elimination of the bacterium from the spleens and livers of anti-TNF antibody-treated mice was delayed, depending on the dose of the antibody injected. Endogenous TNF seemed to be produced early in infection, because suppression of antilisterial resistance was significant when a single injection of anti-TNF antibody was given between day zero and day 2 of infection. The effect of endogenous TNF on antilisterial resistance was due to neither regulation of alpha interferon (IFN-α) and IFN-γ production nor induction of IFN-β subtype 1 (IFN-β_1), because anti-TNF antibody treated-mice produced normal levels of IFN-α and IFN-γ in the bloodstream during infection and administration of monoclonal anti-murine IFN-β_1 antibody had no effect on the development of listeriosis. Alternatively, the listericidal activity of peritoneal macrophages of *L. monocytogenes*-infected mice could be abrogated by injection of anti-TNF antibody in vivo. These results suggest that the lower level of TNF is produced endogenously in mice that received *L. monocytogenes* infection and that it plays an essential role in the host defense against *L. monocytogenes* infection.

Acquired cellular immunity to the facultative intracellular bacterium *Listeria monocytogenes* is mediated by a population of specifically sensitized T cells (18, 29). Activated macrophages are the principal effector cells of resistance, and they acquire their microbiological activities after stimulation with lymphokines released from specific T cells (20). Among T-cell products, participation of gamma interferon (IFN-γ) in antilisterial resistance has been well characterized (5, 11, 13, 14, 25, 27). Although IFN-γ production is below the detection limit in the sera of *L. monocytogenes*-infected mice at all stages of infection, we reported that significant IFN-γ production could be induced in the bloodstream in response to specific antigen late in infection (25, 27). Buchmeier and Schreiber (5) demonstrated the existence and role of the undetectable level of endogenously produced IFN-γ during *L. monocytogenes* infection by showing that inhibition of the generation of activated macrophages and a higher mortality occurred in mice treated with monoclonal anti-murine IFN-γ antibody. We reported that in addition to IFN-γ, IFN-α endogenously produced mainly by natural killer cells in the early stage of infection might play a key role as a messenger in generating antigen-specific T cells involving IFN-γ production and acquired resistance to the infection (25).

Tumor necrosis factor (cachectin) (TNF-α), a protein produced mainly by macrophages, has diverse actions on a wide variety of cells throughout the body, including leukocytes, tumor cells, and fibroblasts, and has profound and important biological effects in the intact host (2, 28). The intimate interaction between TNF and IFN-γ has been reported. For example, TNF and IFN-γ have a synergistic effect on the enhancement of functions of macrophages and polymorphonuclear neutrophils (8, 32). This synergy is probably due to the induction of both TNF receptor expression and the accumulation of mRNA for TNF by IFN-γ (6, 35). Furthermore, the increased expression of major histocompatibility complex class I antigens by TNF is reported to be mediated by TNF-induced IFN-β subtype 1 (IFN-β_1) or IFN-β_2–B cell stimulatory factor (BSF)-2–interleukin-6 (IL-6) (19, 21). Hence TNF as well as IFNs may participate in the cytokine network during immune responses. Havell (10) recently showed that TNF production occurred in vivo and in vitro in *L. monocytogenes*-infected mice and that injection of anti-murine recombinant TNF-α (rTNF-α) immunoglobulin G into sublethally infected mice resulted in increased bacterial growth in the organs and ultimately in death from listeriosis. We focused our studies on in vivo analysis of an endogenous TNF-mediated host defense mechanism, including its effect on the IFN system.

In the present communication, we report that suppression of antilisterial resistance by administration of anti-murine rTNF-α immunoglobulin is due to inhibition of generation of activated macrophages rather than specific T cells. Furthermore, we demonstrated that the effect of endogenous TNF on antilisterial resistance is mediated neither by modulation of IFN-α or IFN-γ production nor by induction of IFN-β_1.

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 previously described (26). 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).

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Anti-TNF-α antibody. An immunoglobulin fraction, which was prepared and purified by $(NH_4)_2SO_4$ precipitation from the serum of a rabbit hyperimmunized with purified murine rTNF-α, was donated by Suntory Institute for Biomedical Research, Osaka, Japan. The antibody (1 mg) neutralized 2.4 × 10^5 U of the cytolytic activity of murine rTNF-α but affected neither the cytolytic activity of human rTNF-α nor the antiviral activities of murine rIFN-γ, murine rIFN-β_1, and partially purified murine natural IFN-α/β. To deplete endogenous TNF in vivo, we gave each mouse a single intravenous injection of different concentrations of the immunoglobulin fraction (0.1 ml) diluted with PBS 2 h before *L. monocytogenes* infection. Normal rabbit globulin used as a control was also prepared by $(NH_4)_2SO_4$ fractionation from the serum of a nonimmunized rabbit.

Anti-murine IFN-β_1 antibody. Rat IgG1 monoclonal antibody against the purified preparation of murine natural IFN-β_1, which was purified by $(NH_4)_2SO_4$ precipitation from the ascites fluid in BALB/c nude mice injected with hybridoma 7F-D3, was donated by Y. Watanabe and Y. Kawade, Institute for Virus Research, Kyoto University, Kyoto, Japan. Mice were injected intravenously with the antibody (0.1 ml), which neutralized 10^5 international units (IU) of murine rIFN-β_1, 2 h before *L. monocytogenes* infection.

Induction of TNF. TNF was induced by injecting mice intravenously with 0.2 ml of a solution containing 25 μg of lipopolysaccharide (LPS) at the desired period after *L. monocytogenes* infection, and the sera were obtained 2 h later. *Salmonella typhimurium* LPS purified by the method of Westphal and Jann (39) was purchased from Difco Laboratories, Detroit, Mich.

Induction of IFNs. IFN-α activity was determined by using the serum specimens obtained from mice 48 h after *L. monocytogenes* infection. IFN-γ was induced by intravenous injection of 50 μg of *Listeria* cell wall fraction (LCWF) into mice on day 5 of *L. monocytogenes* infection, and the sera were collected 6 h later. LCWF was prepared as previously described (22, 25). Briefly, *L. monocytogenes* cells grown in tryptic soy broth (Difco) were washed three times with 0.85% saline and suspended in double-distilled water to make a 20% cell suspension (wet weight/vol). The cells were sonically disrupted and centrifuged at 2,000 × *g* for 20 min. The resulting supernatant was further centrifuged at 14,500 × *g* for 60 min. The precipitate obtained was washed three times with double-distilled water. The LCWF obtained was lyophilized, weighed, reconstituted with 0.85% saline to a concentration of 2.5 mg/ml, and autoclaved. All preparations were carried out under aseptic conditions, and the 0.85% saline and double-distilled water used were sterile and nonpyrogenic. The LCWF was stored at −70°C.

Preparation of samples for TNF and IFN assays. Samples for TNF and IFN assays consisted of pooled sera and extracts of spleen homogenate obtained from at least four mice in each group. Extracts of spleen homogenate were prepared as follows: the spleen was aseptically removed, suspended in 1 ml of 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), and homogenized with a Dounce tissue grinder. The spleen homogenate was frozen and then thawed and clarified by centrifugation at 2,000 × *g* for 10 min. The extracts obtained were sterilized by filtration through a membrane filter (pore size, 0.2 nm; Gelman Sciences, Inc., Ann Arbor, Mich.) and assayed immediately for TNF and IFN.

TNF assay. TNF titers were determined by the cytolytic activity against murine L-929 cells. TNF-sensitive L-929 cells were donated by N. Sato, Department of Biochemistry, National Defense Medical College, Tokorozawa, Japan. Samples were serially diluted, and 1.0 ml of each dilution was inoculated into 96-well flat-bottom microplates (type 25860; Corning Glass Works, Corning, N.Y.) in duplicate. Cells (2 × 10^4) from the cell suspension were added to each well, and the plate was incubated at 37°C in a 5% CO_2 incubator for 48 h. The cells were fixed and stained with 0.5% gentian violet–5% Formalin–50% ethanol in saline for 10 min. The stained plate was washed extensively with running water and dried. Viable cells retained the dye. The dye was solubilized with ethylene glycol monomethyl ether, and the A_{550} of each well was read with a dual-wavelength microplate photometer (MT-22; Corona Electric, Ibaraki, Japan). Titers are expressed as the reciprocal of the dilution of the sample in which 50% of the cells in the monolayer were lysed. Human rTNF-α was included in each assay as a laboratory standard. Human rTNF-α was donated by Suntory Institute for Biomedical Research.

Neutralization of TNF by anti-TNF antibody. The samples were serially diluted twofold, and equal volumes of diluted rabbit anti-murine rTNF-α immunoglobulin, which completely neutralized 1,000 U of murine rTNF-α, were added to each diluent. The mixtures were incubated at 37°C for 1 h before being assayed for TNF activities. Murine rTNF-α was donated by Suntory Institute for Biomedical Research.

Assay and characterization of IFN. 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 (23). Neutralization tests with monoclonal anti-murine IFN-β_1 antibody, monoclonal anti-murine IFN-α antibody, and monoclonal anti-murine IFN-γ antibody were carried out as reported elsewhere (24).

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.

Bactericidal assay. Listericidal activity of peritoneal macrophages was determined by the method of Van Furth and Van Zwet with some modification (38). Briefly, mice which had been injected intravenously with rabbit anti-murine TNF-α immunoglobulin (50 μg) or normal rabbit globulin (50 μg) 2 h earlier were infected intravenously with 2 × 10^4 CFU of *L. monocytogenes* cells and injected intraperitoneally with 3 ml of thioglycolate medium (Eiken Chemical Inc., Tokyo, Japan) on day 3 of infection. The third group contained the uninfected controls, which received PBS only on day zero and thioglycolate medium on day 3 after injection of normal rabbit globulin. Peritoneal exudate cells (PEC; 5 × 10^6) collected on day 7 of the globulin treatment were mixed with 2 × 10^7 viable *L. monocytogenes* cells in RPMI 1640 medium supplemented with 10% fresh homologous serum. The mixture was then incubated at 37°C in a CO_2 incubator for 30 min to allow phagocytosis of the bacteria. To remove the extracellular bacteria, we washed the cell suspension three times by centrifugation at 400 × *g* for 5 min after incubation. The cells containing ingested bacteria were suspended in RPMI 1640 medium supplemented with 10% homologous serum, and then the infected cells (10^6 cells) were inoculated into each well of 96-well flat-bottom microplate. After 0 and 2 h of incubation at 37°C in a CO_2 incubator, the number of viable bacteria remaining in the cells of each well was determined by culturing on

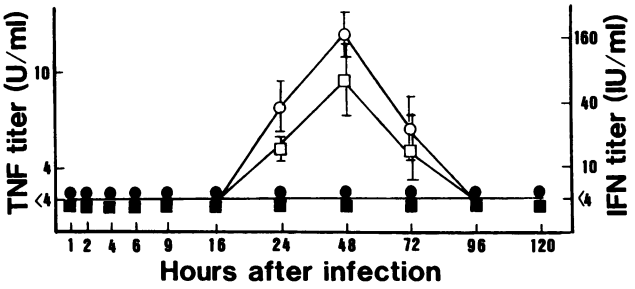

FIG. 1. Production of TNF and IFN in the bloodstream and spleens of mice following *L. monocytogenes* infection. Mice were injected intravenously with 2×10^4 CFU of *L. monocytogenes* cells, and blood and spleens were taken at different times postinfection. The TNF activities of the sera (●) and the spleen extracts (■) and the IFN activities of the sera (○) and the spleen extracts (□) were determined. Each point represents the mean ± standard deviation of the pooled samples (four to five mice) from three experiments.

tryptic soy agar after the cells had been disrupted with cold distilled water.

Statistical evaluation of the data. Data were expressed as mean ± standard deviation, and Student's *t* test was used to determine the significance of the differences between control and experimental groups. The χ^2 test was used to determine the significance of differences in survival rates.

RESULTS

TNF production in *L. monocytogenes*-infected mice in vivo. After mice were infected intravenously with 2×10^4 CFU of *L. monocytogenes*, TNF and IFN activities of both the pooled sera and the spleen extracts obtained from four or five mice were monitored at various times (Fig. 1). Although IFN-α appeared in both the bloodstream and the spleen from 24 to 72 h after infection, no TNF activity was detectable in any of the samples examined. We then tried to induce TNF production in the bloodstream of *L. monocytogenes*-infected mice. LPS (25 μg) was injected intravenously into mice on different days after infection, and serum samples were collected 2 h later (Fig. 2). Biphasic peaks of TNF production induced by LPS were shown early (day 1) and late (day 5) in infection. The cytolytic activities of the serum specimens obtained on both days 1 and 5 of infection were completely neutralized with rabbit anti-murine TNF-α immunoglobulin (data not shown). On the other hand, the specific antigen, LCWF (20 to 500 μg), did not possess the ability to induce TNF in the bloodstream of mice at any stage of infection, although the antigen could induce IFN-γ late in infection (25, 27).

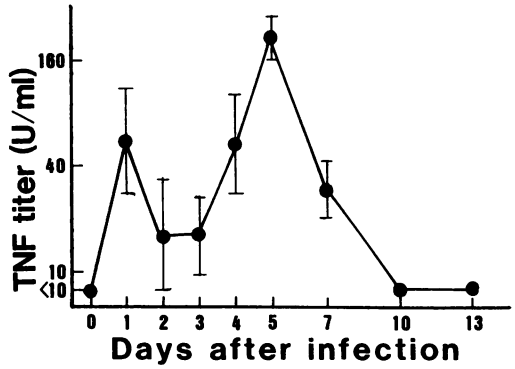

FIG. 2. TNF responses to LPS in the bloodstream of mice during *L. monocytogenes* infection. After mice were infected intravenously with 2×10^4 CFU of *L. monocytogenes* cells, 25 μg of LPS was injected intravenously into them on different days after infection. Serum samples were collected 2 h later and assayed for TNF activities. Each point represents the mean ± standard deviation of the pooled samples (four to five mice) from three experiments.

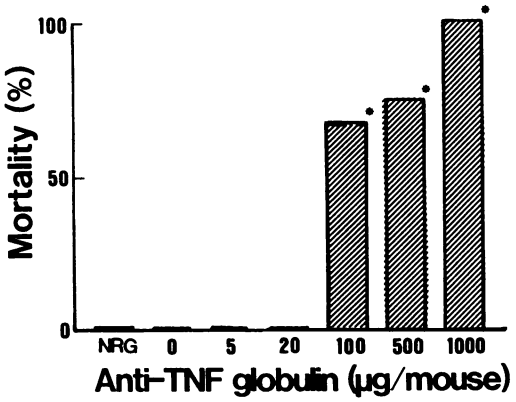

FIG. 3. Cumulative mortality of *L. monocytogenes*-infected mice receiving anti-TNF-α antibody. Different doses of rabbit anti-murine TNF-α immunoglobulin or normal rabbit globulin (NRG; 1,000 μg) were injected intravenously into mice 2 h before infection with 2×10^4 CFU of *L. monocytogenes* cells, which is equivalent to 0.1 50% lethal dose for normal mice, and their survival was observed until day 7 of infection. Each result represents a group of 15 mice from three experiments. Symbol: *, shows a significant difference from the value for untreated mice ($P < 0.01$).

In vivo administration of anti-TNF antibody blocks antilisterial resistance. To determine the existence and mode of action of endogenous TNF during *L. monocytogenes* infection, mice were injected intravenously with different doses of rabbit anti-murine TNF-α immunoglobulin or normal rabbit globulin (1 mg) 2 h before infection with a sublethal dose of the bacterium (2×10^4 CFU; 0.1 50% lethal dose). Although no mouse that received less than 20 μg of the antibody or normal rabbit globulin died, injection of higher doses of the antibody resulted in the death of *L. monocytogenes*-infected mice, and the survival rate decreased as the dose of antibody increased (Fig. 3). Every mouse that had received 1 mg of the antibody died. The antibody-injected mice always died on day 4 or 5 of infection. On the other hand, the uninfected mice were never killed by the injection of 1 mg of the antibody. To confirm that mice that received the antibody would develop listeriosis, the number of *L. monocytogenes* cells in the spleens and livers of these mice was determined early (day 2) and late (day 5) in infection. The growth of the bacterial cells in both organs of anti-TNF immunoglobulin-treated mice was not significantly different from that in normal rabbit globulin-injected animals or untreated controls in the early stage of infection ($P > 0.05$) (Fig. 4). In contrast, although every mouse injected with 20 μg of anti-TNF immunoglobulin died, elimination of bacteria from the spleen and liver was significantly blocked ($P < 0.01$) (Fig. 5). More organisms were detected in both organs of mice that received either 100 or 500 μg of the antibody, even when they escaped death ($P < 0.001$).

Effect of timing of administration of anti-TNF antibody on antilisterial resistance. To investigate when TNF would be produced endogenously and would affect antilisterial resis-

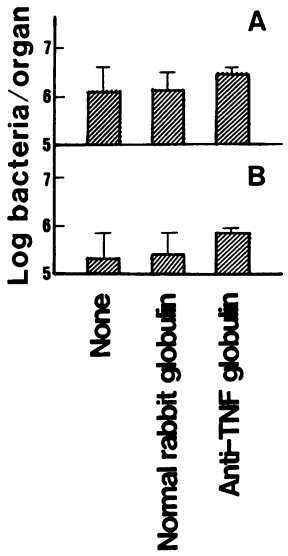

FIG. 4. Growth of *L. monocytogenes* cells in the spleens (A) and livers (B) early in infection of mice that received anti-TNF-α antibody. *L. monocytogenes* cells (2×10^4 CFU) were infected intravenously into mice which had received normal rabbit globulin (1 mg) or anti-TNF-α immunoglobulin (1 mg) 2 h earlier or into untreated controls. 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 separate experiments.

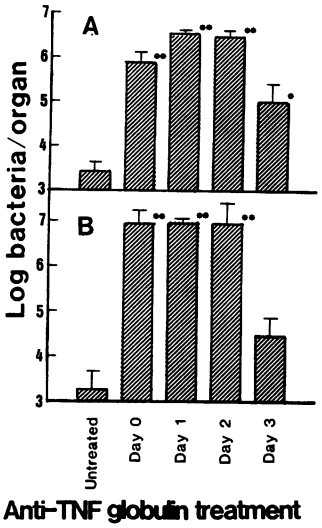

FIG. 6. Estimation of antilisterial resistance in mice by administration of anti-TNF-α antibody on different days of infection. Mice were infected intravenously with 2×10^4 CFU of *L. monocytogenes* cells, and a single injection of rabbit anti-murine TNF-α immunoglobulin was carried out 2 h before or 1, 2, or 3 days after infection. The numbers of bacteria in the spleens (A) and livers (B) were estimated 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. Symbols: * and **, significant difference from the value for untreated mice at $P < 0.01$ and $P < 0.001$, respectively.

tance, a single injection of anti-TNF immunoglobulin (50 μg) 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 (Fig. 6).

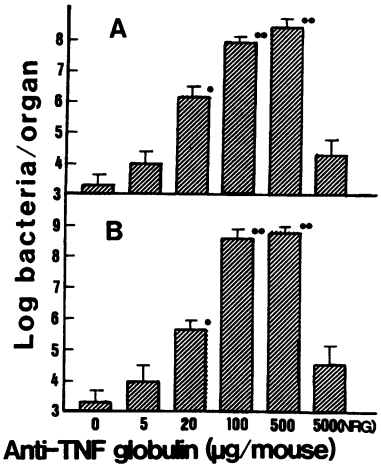

FIG. 5. Growth of *L. monocytogenes* cells in the spleens (A) and livers (B) late in infection in mice that received anti-TNF-α antibody. *L. monocytogenes* cells (2×10^4 CFU) were infected intravenously into mice which had received different doses of rabbit anti-murine TNF-α immunoglobulin or normal rabbit globulin (NRG; 500 μg) 2 h earlier. 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 separate experiments. Symbols: * and **, significant difference from the value for untreated mice at $P < 0.01$ and $P < 0.001$, respectively.

Suppression of the elimination of bacteria from both organs was observed when mice had received a single injection of the antibody on day zero, 1, or 2 of infection ($P < 0.001$). In contrast, administration of anti-TNF immunoglobulin on day 3 of infection had less effect on the elimination of bacteria in the spleen ($P < 0.05$) and liver ($P > 0.05$).

Effect of administration of anti-TNF antibody on listericidal activity of peritoneal macrophages obtained from *L. monocytogenes*-infected mice. We investigated whether administration of anti-TNF immunoglobulin would interfere with the induction of activated macrophages during *L. monocytogenes* infection. Mice which had been injected with anti-TNF immunoglobulin (50 μg) or normal rabbit globulin (50 μg) were infected with 2×10^4 CFU of *L. monocytogenes* cells. Animals in the third group were injected with normal rabbit globulin (50 μg) without being infected. All groups of mice were stimulated with thioglycolate medium 3 days later. PEC were collected on day 7 of the globulin treatment, and their listericidal activities were estimated in vitro (Table 1). Listericidal activity of PEC obtained from *L. monocytogenes*-infected mice with normal rabbit globulin treatment was significantly higher than that in the uninfected controls ($P < 0.01$). However, augmentation of the activity induced by the infection was significantly suppressed when mice were injected with anti-TNF immunoglobulin.

Effect of administration of anti-TNF antibody on IFN-α and IFN-γ production in the bloodstream of *L. monocytogenes*-infected mice. Endogenously produced IFN-α and IFN-γ are both essential to the establishment of antilisterial resistance (1, 5, 25, 27). Therefore, we investigated the possibility that endogenously produced TNF would regulate endogenous production of IFN-α or IFN-γ or both during *L. monocytogenes* infection. IFN-α can be detected in the bloodstream on day 2 of infection, and IFN-γ production in vivo can be

TABLE 1. Effect of in vivo administration of anti-TNF-α antibody on in vitro listericidal activity of thioglycolate-elicted peritoneal macrophages

Expt no.	Macrophages from mice pretreated with[a]:		No. of viable intracellular *L. monocytogenes* cells (10^4)[b] at:	
	Globulin	*L. monocytogenes*	0 h	2 h
1	Normal rabbit	−	614 ± 69	571 ± 42
	Normal rabbit	+	571 ± 42	150 ± 59[c]
	Anti-TNF-α	+	563 ± 74	343 ± 63[d]
2	Normal rabbit	−	314 ± 75	275 ± 36
	Normal rabbit	+	263 ± 58	85 ± 20[c]
	Anti-TNF-α	+	275 ± 63	195 ± 56

[a] Mice were injected intravenously with normal rabbit globulin (50 μg) or rabbit anti-TNF-α immunoglobulin (50 μg) 2 h before being infected intravenously with 2×10^4 CFU of *L. monocytogenes* cells or injected with PBS only; they were given thioglycolate medium intravenously 3 days later. PEC were harvested on day 7 of the globulin treatment.

[b] PEC (5×10^6) were incubated with 2×10^7 viable *L. monocytogenes* cells for 30 min, washed to remove nonphagocytized cells, and then incubated for 2 h at 37°C in a CO_2 incubator. Each result represents the mean CFU ± standard deviation for triplicate macrophage cultures.

[c] Significantly different from value for 0 h ($P < 0.01$).

[d] Significantly different from value for 0 h ($P < 0.05$).

amplified by stimulation of specific antigen, LCWF, late in infection (25). After mice that had been injected with 50 μg of anti-TNF immunoglobulin or 50 μg of normal rabbit globulin 2 h earlier or untreated controls were infected intravenously with *L. monocytogenes*, IFN-α activity in the sera obtained from them on day 2 of infection was determined. Furthermore, other mice in each group were injected intravenously with LCWF (50 μg) on day 5 of infection, and the sera for IFN-γ titrations were taken 6 h later (Fig. 7). The titers of both IFN-α and IFN-γ in sera obtained from anti-TNF immunoglobulin-treated mice were comparable to those in sera obtained from both the untreated controls and the normal rabbit globulin-treated group.

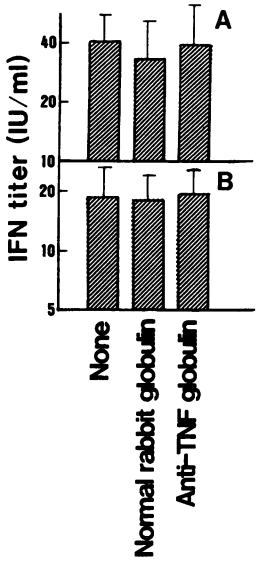

FIG. 7. Effect of in vivo administration of anti-TNF-α antibody on production of IFN-α (A) and IFN-γ (B) in the bloodstream of mice during *L. monocytogenes* infection. *L. monocytogenes* cells (2×10^4 CFU) were injected intravenously into mice which had received rabbit anti-murine TNF-α immunoglobulin (50 μg) or normal rabbit globulin (50 μg) 2 h earlier or into untreated controls. The sera for IFN-α assay were collected 48 h later. IFN-γ was induced by injection of LCWF (50 μg) on day 5 of infection, and the sera were taken 6 h later. Each result represents the mean ± standard deviation of the pooled samples (four mice) from three experiments.

Effect of administration of monoclonal anti-murine IFN-β_1 antibody on antilisterial resistance. Some of the biological activities of TNF are reported to be due to TNF-induced IFN-β_1 or IFN-β_2–BSF-2–IL-6 (19, 21). Therefore, we investigated whether the effect of TNF on antilisterial resistance would be mediated by the IFN-β family. Monoclonal anti-murine IFN-β_1 antibody or PBS was injected into mice 1 h before infection, and the number of *L. monocytogenes* cells in the spleens and livers of the mice was determined on days 2 and 5 of infection (Fig. 8). Anti-IFN-β_1 antibody had no effect on the elimination of bacteria from either organ at any stage of infection.

DISCUSSION

The studies presented here demonstrate that TNF is produced endogenously and plays an essential role in host defense against *L. monocytogenes* infection. Administration of rabbit antibody against murine rTNF-α to the infected mice inhibited the generation of activated macrophages, abrogated the clearance of bacteria from the spleens and livers, and, finally, resulted in mortal listeriosis.

Administration of anti-TNF immunoglobulin at higher doses resulted in lethal *L. monocytogenes* infection, even though the infectious dose was sublethal (Fig. 3). The immunoglobulin preparation showed no lethal toxicity to uninfected mice. The increase in bacterial growth in the spleens and livers of anti-TNF immunoglobulin-treated mice was demonstrated in the late stage of infection, depending on the dose of antibody injected (Fig. 5). On the other hand, no endotoxin was detected in a solution of anti-TNF immuno-

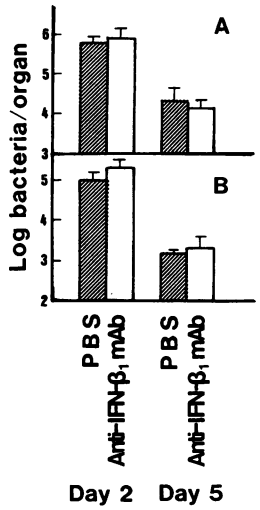

FIG. 8. Effect of in vivo administration of monoclonal anti-murine IFN-β_1 antibody on the growth of *L. monocytogenes* cells in the spleens (A) and livers (B) of mice early (Day 2) and late (Day 5) in infection. The mice were injected intravenously with 0.1 ml of monoclonal antibody containing 10^4 neutralizating units or PBS and received 2×10^4 CFU of bacteria 2 h later. The number of bacterial cells in the organs was estimated on days 2 and 5 of infection. Each result represents the mean ± standard deviation for a group of four mice. The results were reproduced in two different experiments.

globulin by a *Limulus* amebocyte lysate assay (data not shown), although LPS, even at trace levels, is known to modulate immune responses (7). The results suggest that anti-TNF immunoglobulin suppresses antilisterial resistance by neutralizing endogenous TNF.

We showed that administration of anti-TNF immunoglobulin until day 2 of infection resulted in a decrease in antilisterial resistance, but no decrease occurred when the antibody was injected thereafter (Fig. 6), suggesting that endogenous TNF might be produced and act on the antilisterial defense in the early stage of infection. In fact, Havell (10) reported that TNF could be detected in the bloodstream of lethally infected mice on day 1 of *L. monocytogenes* infection. We also showed that the first peak of TNF production induced by LPS in the bloodstream of sublethally infected mice was seen on day 1 of infection (Fig. 2). In contrast, depletion of endogenous TNF in *L. monocytogenes*-infected mice by injection of the antibody resulted in inhibition of the antigen-specific elimination of bacteria from the spleens and livers late rather than early in infection (Fig. 4 and 5).

Antilisterial resistance acquired late in infection is mediated by antigen-specific T cells (18, 20, 29). Kaufmann et al. (15, 16) reported that antigen-specific $L3T4^+$ and $Lyt2^+$ T cells are involved in protection against *L. monocytogenes* infection in mice during infection. Both subpopulations of antigen-specific helper and cytolytic T cells can produce IFN-γ by stimulation with a specific antigen (11, 13, 14, 25). Hence, we estimated the ability of *L. monocytogenes*-infected mice, which had received anti-TNF immunoglobulin, to produce IFN-γ in response to specific antigen in the bloodstream as a parameter of functions of sensitized T cells (Fig. 7). The titers of IFN-γ in the bloodstream of anti-TNF immunoglobulin-treated mice was comparable to the antiviral activities in the untreated controls and normal rabbit globulin-treated animals. Therefore, it is unlikely that endogenous TNF might affect functions of antigen-specific T cells. On the other hand, macrophages activated by antigen-specific T-cell-derived lymphokines are essential to the complete elimination of *L. monocytogenes* cells from the tissues of the host (12, 20). Hence, we assumed another possibility, i.e., that endogenous TNF participates in activation of macrophages. In fact, we demonstrated that in vitro listericidal activity of peritoneal macrophages obtained from *L. monocytogenes*-infected mice which had received anti-TNF immunoglobulin was significantly lower than that of macrophages from the animals without the antibody treatment (Table 1), suggesting that endogenous TNF might participate in activation of macrophages. However, Buchmeier and Schreiber (5) and Bancroft et al. (1) reported that neutralization of endogenous IFN-γ by injection of monoclonal anti-murine IFN-γ antibody resulted in inhibition of macrophage activation in *L. monocytogenes*-infected mice, although Van Dissel et al. (37) recently reported that murine rIFN-γ failed to activate antilisterial activity of macrophages either in vivo or in vitro. Therefore, the question arose that endogenous TNF might modulate production of IFN-γ and/or another IFN, IFN-α, which might play a key role as a messenger to generate antigen-specific T cells involving IFN-γ production (25). However, this is unlikely, because administration of anti-TNF immunoglobulin affected production of neither IFN-α nor IFN-γ in the bloodstream of *L. monocytogenes*-infected mice (Fig. 7). On the other hand, another possibility arose, i.e., that IFN-β_1 or IFN-β_2–BSF-2–IL-6 induced by endogenous TNF acts substantially as an essential factor in antilisterial resistance, since it has been reported that some biological activities of TNF involving antiviral action and major histocompatibility complex class I expression might be mediated by TNF-induced IFN-β_1 and/or IFN-β_2–BSF-2–IL-6 (17, 19, 21, 36). Therefore, we investigated the effect of anti-murine IFN-β_1 monoclonal antibody injection on antilisterial resistance (Fig. 8). Our result suggests that the effect of endogenous TNF on antibacterial defense is independent on IFN-β_1, although we could not eliminate a possible role of IFN-β_2–BSF-2–IL-6.

On the basis of these observations, our present studies suggest that TNF produced by macrophages, as well as IFN-γ produced by T cells, is an essential factor in the host defense against *L. monocytogenes* infection. Although our data demonstrate that TNF might not modulate the IFN-producing system and could act even without cooperation with IFNs, it is unlikely that TNF and IFNs participate in antilisterial resistance independently, because the intimate interaction between TNF and IFN-γ involving enhancement of TNF production by IFN-γ and their synergy in various biological activities have been demonstrated (4, 6, 8, 31, 32, 34, 40, 41). We are now studying the host defense mechanism against *L. monocytogenes* infection by focusing on the interaction between TNF and IFN-γ endogenously produced.

Participation of endogenous TNF as cachectin in pathogenesis of microbial infections has been reported. Administration of antibodies against TNF-α (cachectin) protects the host from the lethal effect of endotoxin (3), from septic shock during lethal infection by *Escherichia coli* (33), and from cerebral malaria (9). Furthermore, it was shown that injection of anti-TNF immunoglobulin G prevents graft-versus-host disease (30). In contrast, endogenous TNF protects the host from lethal infection by *L. monocytogenes* as demonstrated herein and by Havell (10), showing that TNF possesses opposite actions to those of the host. Hence, monitoring and modulating endogenous TNF might be important in treating various diseases, including infectious diseases.

ACKNOWLEDGMENTS

We thank H. Ito for technical assistance.

This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, and Culture.

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