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Endogenous Interleukin-4, but Not Interleukin-10, Is Involved in Suppression of Host Resistance against *Listeria monocytogenes* Infection in Gamma Interferon-Depleted Mice

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The production and roles of endogenous interleukin-4 (IL-4) and IL-10 in a sublethal infection with *Listeria monocytogenes* were studied in normal mice and anti-gamma interferon (IFN-γ) monoclonal antibody (MAb)-pretreated mice. In normal mice, the expression of mRNAs for IL-4 and IL-10, which was amplified by reverse transcription-PCR, was induced in the spleens and livers either early or late in infection, although the serum IL-4 and IL-10 were not detectable by enzyme-linked immunosorbent assays. In vivo administration of anti-IL-4 MAb showed no effect on antilisterial resistance, whereas anti-IL-10 MAb partially diminished the defense. In anti-IFN-γ MAb-pretreated mice, a delay in the bacterial elimination from the spleens and livers was observed and high titers of serum IL-4 and IL-10 were induced late in infection. Production of endogenous IL-4 and IL-10 was suppressed in both $CD4^+$ cell- and $CD8^+$ cell-depleted mice. The suppression of antilisterial resistance in anti-IFN-γ MAb-pretreated mice was canceled when anti-IL-4 MAb was injected late in infection, whereas anti-IL-10 MAb showed no effect. These results suggest that the host immune responses were polarized into the T-helper 2 phenotype in anti-IFN-γ MAb-pretreated mice and that inhibition of host resistance against *L. monocytogenes* by depletion of IFN-γ might be attributable to IL-4 produced by T cells polarized into the T-helper 2 phenotype as well as the inhibition of the IFN-γ effects.

Host resistance against *Listeria monocytogenes*, a facultative intracellular pathogen, is controlled by cell-mediated immunity and regulated by endogenous cytokines. Some cytokines can be divided into two groups, namely, T-helper 1 (Th1)-derived cytokines and Th2-derived cytokines, which regulate the action and production of each other (22). *L. monocytogenes* promotes the induction of the Th1 response, including gamma interferon (IFN-γ) production (7, 14). In parallel with the growth of *L. monocytogenes* in the spleens and livers of mice, endogenous IFN-γ can be detected in the bloodstreams and organs (26, 28). Studies including in vivo administration of anti-IFN-γ monoclonal antibodies (MAbs) and gene knockout technology demonstrated that IFN-γ plays a crucial role in antilisterial resistance (1, 3, 11, 15). On the other hand, the regulation of the host resistance by Th2 cytokines including interleukin-4 (IL-4) and IL-10 has been reported. IL-4-antagonizes antilisterial resistance (10, 36). IL-10 was also reported to inhibit antilisterial resistance (9, 37), although Wagner et al. (40) reported that IL-10 has both beneficial and detrimental effects on it, depending on the phase of infection.

Endogenous IFN-γ production induced by *L. monocytogenes* infection peaks early in infection (26). Our previous studies (25, 26, 28) and that of Dunn and North (5) demonstrated that the early endogenous IFN-γ plays a crucial role in antilisterial resistance. In the late stage, however, Harty et al. (11, 12) reported that $CD8^+$ T-cell-mediated resistance, which is a major mechanism late in infection (21), is IFN-γ independent. In fact, administration of anti-IFN-γ MAb after the early stage of infection showed no effect on antilisterial resistance in mice (25), although severe exacerbation of the infection by IFN-γ depletion is obvious late in infection (5, 25). These results suggest that the role of IFN-γ in antilisterial resistance might not be fully explained by its direct effector functions, e.g., macrophage activation. We assumed that polarization of the Th2 response might occur and that Th2 cytokines, including IL-4 and IL-10, might be involved in the decrease of antilisterial resistance late in infection in IFN-γ-deficient mice. In this study, we demonstrate that high titers of endogenous IL-4 and IL-10 are produced late in *L. monocytogenes* infection in anti-IFN-γ MAb-pretreated mice, in which the elimination of the bacterium is markedly delayed from the spleens and livers. We further show that endogenous IL-4, but not IL-10, might be responsible for the suppressive effect of anti IFN-γ MAb pretreatment on antilisterial resistance late in infection.

MATERIALS AND METHODS

Mice. Specific-pathogen-free female ddY mice (obtained from S. L. C. Hamamatsu, Shizuoka, Japan), 5 to 7 weeks old, were used.

Bacteria. *L. monocytogenes* 1b 1684 cells were prepared as described previously (24). The concentration of washed cells was adjusted spectrophotometrically at 550 nm. Mice were infected intravenously with 0.2 ml of a solution containing 10^4 CFU of viable *L. monocytogenes* in 0.01 M phosphate-buffered saline (PBS; pH 7.4).

Determination of the number of viable *L. monocytogenes* bacteria in the organs. The number of viable *L. monocytogenes* in the spleens and livers of the infected animals was established by plating serial 10-fold dilutions of organ homogenates in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 1% (wt/vol) 3 [(cholamidopropyl)-dimethyl-ammonio]-1-propanesulfate (CHAPS; Wako Pure Chemical Co., Osaka, Japan) on tryptic soy agar (Difco Laboratories, Detroit, Mich.). Colonies were routinely counted 18 to 24 h later.

Preparation of organ extracts for cytokine assays. The spleen homogenates for IFN-γ and IL-4 assays were prepared as follows. The spleens were aseptically removed from the mice and suspended in RPMI 1640 medium containing 1% CHAPS, and 10% (wt/vol) homogenates were prepared with a Dounce grinder

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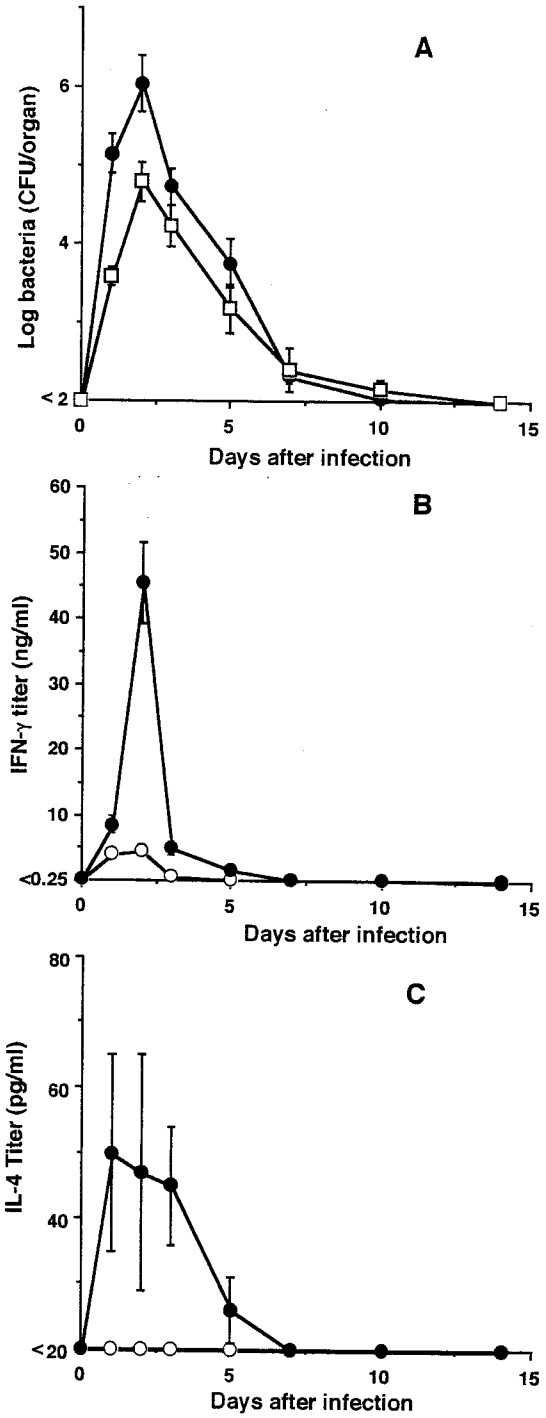

FIG. 1. Production of endogenous IFN-γ (B) and IL-4 (C) in the bloodstreams (○) and spleens (●) of *L. monocytogenes*-infected mice and the numbers of bacteria (A) in the spleens (●) and livers (□). Each point represents the mean ± standard deviation for a group of five mice.

and then clarified by centrifuging at 2,000 × *g* for 20 min (28). The spleen extracts were stored at −80°C until the cytokine assays were performed.

IFN-γ assay. The IFN-γ assay was carried out by a double-sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (26). Purified rat anti-mouse IFN-γ MAb produced by hybridoma R4-6A2 and rabbit anti-recombinant mouse IFN-γ serum (26) were used for the ELISA. All ELISAs were run with recombinant mouse IFN-γ produced and purified by Genentech, Inc., San Francisco, Calif.

IL-4 and IL-10 assays. IL-4 and IL-10 were determined by ELISAs. Microtiter plates were coated with 200 ng of rat anti-mouse IL-4 MAb (11B11; PharMingen, San Diego, Calif.) or rat anti-mouse IL-10 MAb (JES5-2A5; PharMingen) in 0.05 M carbonate buffer (pH 9.6). Uncoupled binding sites in the wells were blocked with PBS containing 2.5% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The wells were incubated with 100 μl of a sample in triplicate overnight at 4°C and then exposed to 50 ng of biotinylated rat anti-mouse IL-4 MAb (BVD6-24G2; PharMingen) or biotinylated rat anti-mouse IL-10 MAb (SXC-1; Phar Mingen) in PBS containing 1% bovine serum albumin. The plate was developed with an avidin-biotinylated peroxidase complex (Vector Laboratories, Burlingame, Calif.) and an *o*-phenylenediamine (Wako) substrate. Selected amounts of recombinant mouse IL-4 (Genzyme Co., Boston, Mass.) or recombinant mouse IL-10 (Genzyme) suspended in 100 μl of PBS containing 1% bovine serum albumin was used to construct a standard curve to accompany each experiment. The assay was determined to detect significant concentrations of IL-4 and IL-10 as low as 20 pg/ml.

RNA extraction. Pieces of spleens and livers (0.05 g each) were suspended in 0.5 ml of solution D (4 M guanidinium isothiocyanate, 0.5% *N*-lauroylsarcosine, 25 mM sodium citrate, 100 mM 2-mercaptoethanol) and homogenized with a Dounce grinder. The homogenate was transferred to a 12-ml polypropylene tube. Subsequently, 50 μl of 2 M sodium acetate (pH 4.0), 500 μl of Tris-EDTA buffer-saturated phenol, and 100 μl of chloroform were added to the lysates, with the mixture being thoroughly vortexed after each addition. The mixtures was then chilled on ice for 30 min and centrifuged at 10,000 × *g* for 15 min at room temperature. The aqueous phase was recovered, and RNA was precipitated with an equal volume of isopropanol and stored at −80°C until further processing. The precipitates were centrifuged at 10,000 × *g* for 15 min, washed once with 75% ethanol, and centrifuged again at 10,000 × *g* for 15 min. The tubes were inverted to air dry the pellets. The pellets were then resuspended in 50 μl of double-distilled water. The RNA yield was determined by the A_{260}.

Reverse transcription-PCR. Preparation of cDNA by reverse transcription was performed in the following way. Total RNA as described above (1 μg in a volume of less than 10 μl) was mixed with 4 μl of reverse transcription buffer (Gibco-BRL, Life Technologies, Inc., Gaithersburg, Md.), 4 μl of 1.25 mM deoxynucleoside triphosphates (Pharmacia Biotechnology AB, Uppsala, Sweden), 0.5 μl of random primer (Takara Shuzo Co., Otsu, Shiga, Japan), and distilled water to make the final volume 19 μl. The mixture was overlaid with 100 μl of mineral oil (Aldrich Chemical Co., Milwaukee, Wis.) and heated at 85°C for 5 min. After cooling on ice, 1 μl (200 U) of Moloney murine leukemia virus reverse tran-

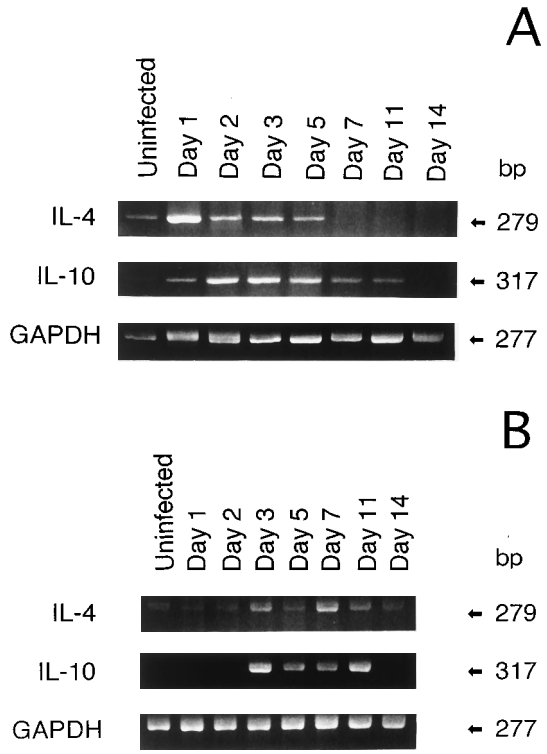

FIG. 2. PCR-assisted mRNA amplification of IL-4, IL-10, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the spleens (A) and livers (B) of mice at various times after *L. monocytogenes* infection.

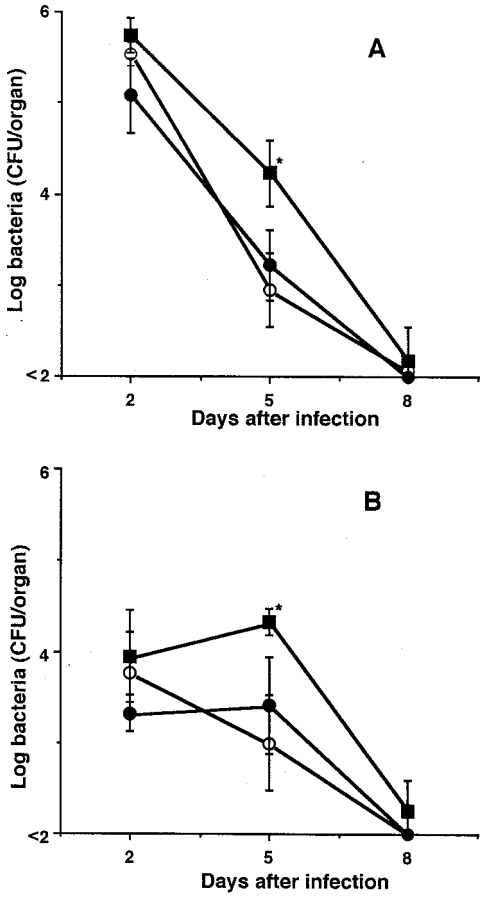

FIG. 3. Effect of in vivo administration of MAbs against IL-4 and IL-10 on the growth of *L. monocytogenes* cells in the spleens (A) and livers (B) of mice. Anti-IL-4 MAb (●), anti-IL-10 MAb (■), or NRG (○) was injected 2 h before infection. Each result represents the mean ± standard deviation for a group of five mice. An asterisk indicates a significant difference from the value for the NRG-injected mice at a P of <0.01.

scriptase (Gibco-BRL) was added. The mixture was incubated at 37°C for 60 min and heated at 85°C for 5 min.

PCR amplification was performed with a Program Temp Control System (PC-700; ASTEC, Inc., Fukuoka, Japan). The reaction mixture consisted of 20 μl of sample cDNA, 8 μl of PCR amplification buffer (Gibco-BRL), 4 μl of 1.25 mM deoxynucleoside triphosphates, 1 μl of 20 mM 5′ and 3′ primers, 0.5 μl (2.5 U) of *Taq* DNA polymerase (Gibco-BRL), and 47 μl of distilled water to make the final volume 60.5 μl. The following oligonucleotides were used: for IL-10, 5′-GCAGGGGCCAGTACAGCCGGGAA-3′ and 5′-CCTCAGCCGCATCCT GAGGGTCTTCAGC-3′; for glyceraldehyde-3-phosphate dehydrogenase, 5′-TGAAGGTCGGTGTGAACGGATTTGG-3′ and 5′-ACGACATACTCAG CACCAGCATCAC-3′. These primers were made to our order by Hokkaido System Science Co., Sapporo, Japan. The primers for IL-4 was purchased from Stratagene, La Jolla, Calif. The predicted sizes of amplified products for IL-4, IL-10, and glyceraldehyde-3-phosphate dehydrogenase were 279, 317, and 277 bp, respectively. One PCR cycle was run under the following conditions: DNA denaturation at 93°C for 29 s, primer annealing at 55°C for 29 s, and DNA extension at 72°C for 2 min. After 35 cycles of amplification, the reaction was terminated.

Agarose gel electrophoresis. The PCR products were analyzed by agarose gel electrophoresis with a horizontal 4.0% agarose gel (NuSieve/SeaKem = 3:1; FMC Bioproducts, Rockland, Maine) in 1× TAE buffer supplemented with 0.005% ethidium bromide for DNA staining. Undiluted PCR products (5 μl) plus 5 μl of bromophenol blue were applied to each well. Gels were run in 1× TAE buffer at 100 V for 50 min. The PCR products were visualized and photographed on a UV transilluminator.

In vivo depletion of endogenous cytokines. Hybridoma cell lines secreting MAbs against mouse IFN-γ (R4-6A2; rat immunoglobulin G1) (34), mouse IL-4 (11B11; rat immunoglobulin G1) (29), and mouse IL-10 (JES5-2A5; rat immunoglobulin G1) (23) were used. MAbs found in the ascites fluid were partially purified by $(NH_4)_2SO_4$ precipitation. The mice were given a single intravenous injection of 1 mg of anti-IFN-γ MAb 2 h before infection, and then they received a single injection of MAbs against IL-4 or IL-10 on day 5 of infection. Normal rat globulin (NRG) was injected as a control for the MAbs. NRG was prepared as described previously (27).

MAbs against cell surface molecules. Hybridoma cell lines GK1.5 (anti-CD4; rat immunoglobulin G2b) and 53-6.72 (anti-CD8; rat immunoglobulin G2a) were used. The MAbs in the ascites fluid were partially purified by $(NH_4)_2SO_4$ precipitation. Mice were given intravenously a single 400-μg injection of anti-CD4 MAb or anti-CD8 MAb on day 5 of *L. monocytogenes* infection. With these schedules and doses, the MAbs could deplete the corresponding cells as described previously (27).

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

Statistical evaluation of the data. Data were expressed as means ± standard deviations, and the Wilcoxon rank sum test was used to determine the significance of the differences of bacterial counts in the specimens between control and experimental groups. Each experiment was repeated at least twice and accepted as valid only when trials showed similar results.

RESULTS

Detection of endogenous IL-4 and IL-10 during *L. monocytogenes* infection. After mice were infected with 10^4 CFU of *L. monocytogenes* cells, IFN-γ, IL-4, and IL-10 in the bloodstreams and spleens were monitored at various times. IFN-γ peaked on day 2 of infection in the bloodstreams and spleens as described previously (Fig. 1B) (26, 28). IL-4 peaked on day 1 and was detected up to day 5 of infection in the spleens, whereas no IL-4 was detectable in any serum samples (Fig. 1C). No IL-10 appeared in the bloodstreams during infection (data not shown). We were unable to detect IL-10 in the

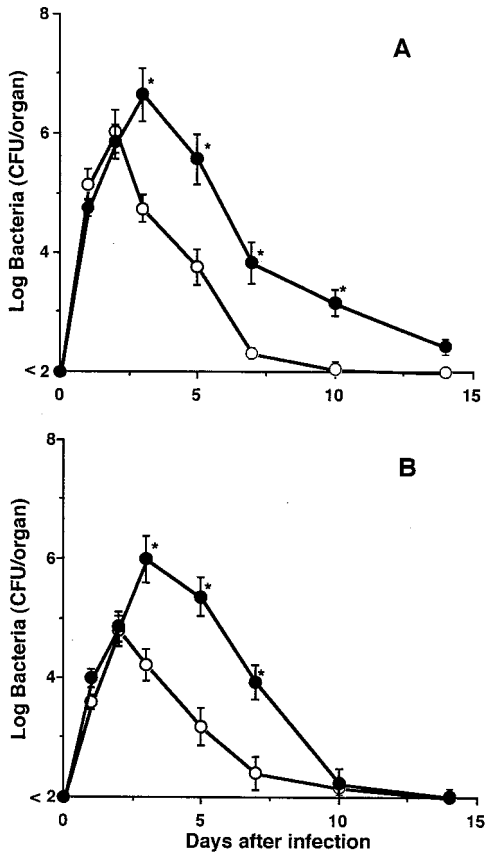

FIG. 4. Effect of in vivo administration of anti-IFN-γ MAb on the growth of *L. monocytogenes* cells in the spleens (A) and livers (B) of mice. Anti-IFN-γ MAb (●) or NRG (○) was injected 2 h before infection. Each result represents the mean ± standard deviation for a group of five mice. An asterisk indicates a significant difference from the value for the NRG-injected mice at a P of <0.01.

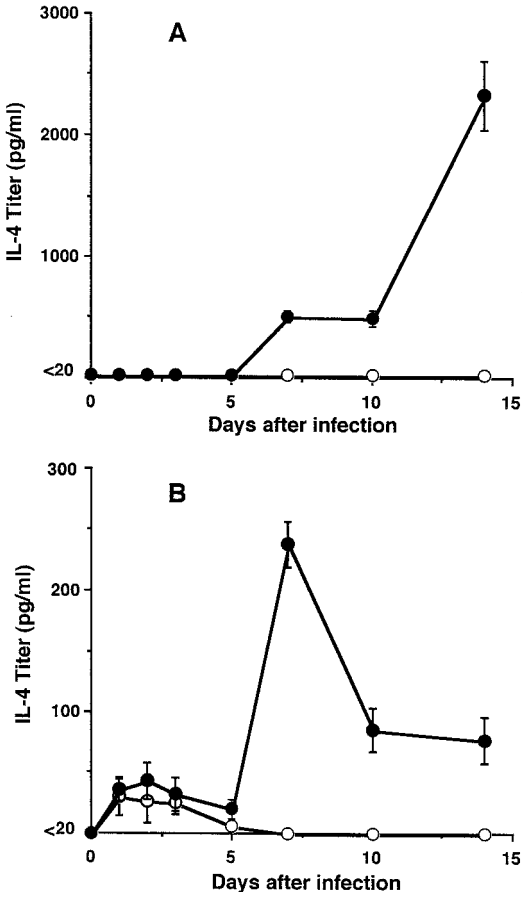

FIG. 5. Endogenous IL-4 production in the bloodstreams (A) and spleens (B) of anti-IFN-γ MAb-pretreated mice (●) and NRG-pretreated mice (○) induced by *L. monocytogenes* infection. Each result represents the mean ± standard deviation for a group of five mice.

spleens possibly because an unknown agent(s) in the spleen extracts interfered with the IL-10 ELISA used herein. Moreover, we failed to determine these cytokines in the liver extracts by the ELISA systems for IL-4 and IL-10 used herein because endogenous biotin in the organ interfered with the assays. Therefore, we decided to detect IL-4 and IL-10 mRNA expression in the spleens and livers by reverse transcription-PCR (Fig. 2). IL-4 and IL-10 mRNAs were induced in the spleens at 24 and 48 h after infection, respectively (Fig. 2A). The obvious expression of mRNAs for both cytokines was observed up to day 5 of infection. The expression of mRNAs for IL-4 and IL-10 was induced in the livers after day 3 of infection (Fig. 2B).

Effects of in vivo administration of MAbs against IL-4 and IL-10 on host resistance against *L. monocytogenes* infection. To determine the mode of action of endogenous IL-4 and IL-10 during *L. monocytogenes* infection, mice were given single intravenous injections of anti-IL-4 MAb, anti-IL-10 MAb, or NRG 2 h before *L. monocytogenes* infection, and the numbers of bacterial cells in the spleens and livers were determined on days 2, 5, and 8 of infection (Fig. 3). The number of bacteria in anti-IL-4 MAb-pretreated mice was lower than that in NRG-treated mice on day 2. However, the difference was insignificant ($P > 0.05$). Thereafter, the fate of bacteria in the organs was similar for both groups. In contrast, the bacterial growth in the spleens and livers of anti-IL-10 MAb-pretreated mice was significantly enhanced on day 5 of infection ($P < 0.01$), whereas the bacterial numbers in the organs of the MAb-pretreated mice were comparable to those in NRG-pretreated mice on either day 2 or day 8.

Endogenous IL-4 and IL-10 production induced by *L. monocytogenes* infection in anti-IFN-γ MAb-pretreated mice. We investigated whether polarization of the Th2 response included endogenous IL-4 and IL-10 production when endogenous IFN-γ was neutralized in vivo by injection of anti-IFN-γ MAb. Mice were given single intravenous injections of anti-IFN-γ MAb or NRG 2 h before *L. monocytogenes* infection, and the numbers of bacterial cells in the spleens and livers were determined at various times (Fig. 4). The bacterial growth was significantly enhanced in anti-IFN-γ MAb-pretreated mice after 3 days compared with that in NRG-pretreated mice. In both groups, IL-4 titers in the serum samples and spleen extracts and IL-10 titers in the serum samples were determined. No IL-4 was detected in the serum specimens of NRG-pretreated mice, whereas IL-4 appeared on day 7 and the highest titer of IL-4 was observed on day 14 of infection in anti-IFN-γ MAb-pretreated mice (Fig. 5A). The low titers of IL-4 were induced on days 1 to 5 of infection in the spleens of anti-IFN-γ MAb-pretreated mice as well as those of NRG-pretreated mice; thereafter, high titers of IL-4 appeared and peaked on day 7 of infection (Fig. 5B). No IL-10 was detected in the specimens of NRG-pretreated mice, whereas IL-10 was induced in the bloodstreams and production peaked on day 5 of infection in anti-IFN-γ MAb-pretreated mice (Fig. 6).

Effect of MAbs against T-cell subsets on endogenous IL-4 and IL-10 production in anti-IFN-γ MAb-pretreated mice. A recent study (32) showed that $CD8^+$ T cells as well as $CD4^+$ T cells can differentiate into Th2 cytokine-producing cells in the presence of IL-4 and anti-IFN-γ MAb. Therefore, we determined the T-cell subsets which are responsible for the production of endogenous IL-4 and IL-10. Anti-IFN-γ MAb-pretreated mice were injected with anti-CD4 MAb, anti-CD8 MAb, or NRG on day 5 of infection. More than 90% of $CD4^+$ or $CD8^+$ cells were depleted in the corresponding MAb-treated mice 24 h later (data not shown). The serum specimens were obtained from these animals on day 7 of infection. IL-4 production was completely inhibited in $CD4^+$ cell-depleted mice (Fig. 7A). Moreover, the IL-4 titers were significantly decreased in $CD8^+$ cell-depleted mice ($P < 0.01$). IL-10 pro-

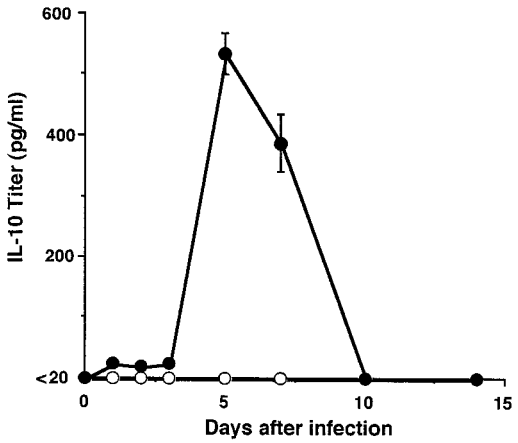

FIG. 6. Endogenous IL-10 production in the bloodstreams of anti-IFN-γ MAb-pretreated (●) and NRG-pretreated (○) mice induced by *L. monocytogenes* infection. Each result represents the mean ± standard deviation for a group of five mice.

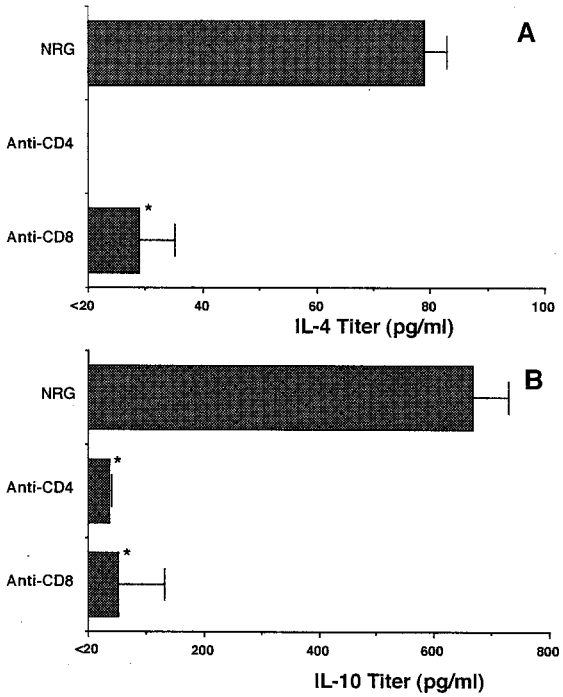

FIG. 7. Effect of in vivo administration of MAbs against T-cell subsets on endogenous IL-4 and IL-10 production induced by *L. monocytogenes* infection in mice which received anti-IFN-γ MAb 2 h before infection. Anti-IFN-γ MAb-pretreated mice were injected with NRG, anti-CD4 MAb, or anti-CD8 MAb on day 5 of infection. The serum samples for IL-10 and IL-4 were taken on day 7. Each result represents the mean ± standard deviation for a group of five mice. An asterisk indicates a significant difference from the value for the NRG-injected mice at a P of <0.01.

duction was also suppressed in either $CD4^+$ or $CD8^+$ cell-depleted mice ($P < 0.01$).

Effect of in vivo administration of MAbs against IL-4 and IL-10 on antilisterial resistance in anti-IFN-γ MAb-pretreated mice. We investigated whether IL-4 and IL-10 are involved in a decrease in antilisterial resistance late in infection. Anti-IFN-γ MAb-pretreated mice or NRG-pretreated mice were injected with anti-IL-4 MAb or NRG on day 5 of infection, and the numbers of bacteria in the spleens and livers of these animals were determined on day 8 of infection (Fig. 8). The number of bacteria in the organs of control mice, which received NRG on day 0, was not affected by anti-IL-4 MAb treatment, whereas the numbers of bacteria in the organs were significantly decreased in anti-IFN-γ MAb-pretreated mice when anti-IL-4 MAb was injected. The bacterial numbers in the mice which were treated with both anti-IFN-γ MAb and anti-IL-4 MAb were comparable to those in the control mice, which received NRG on day 0. Alternatively, anti-IFN-γ MAb-pretreated mice or NRG-pretreated mice were injected with anti-IL-10 MAb instead of anti-IL-4 MAb on day 5 of infection, and the bacterial numbers of the organs were determined on day 8 of infection (Fig. 9). In vivo administration of anti-IL-10 MAb showed no effect on the elimination of *L. monocytogenes* cells from the spleens and livers of either NRG-pretreated mice or anti-IFN-γ MAb-pretreated mice.

DISCUSSION

IL-4 and IL-10 mRNAs were induced in the spleens and livers of *L. monocytogenes*-infected mice (Fig. 2) as reported previously (6, 16, 39). Quantitatively, however, their production was less than IFN-γ production because neither IL-4 nor IL-10 was detected by ELISA in the serum specimens of *L. monocytogenes*-infected mice. Moreover, the peak titer of IL-4 in the spleens was extremely low compared with that of IFN-γ (50 ± 15 pg/ml versus 45.5 ± 6.2 ng/ml) (Fig. 1). The previous studies (10, 36) reported that in vivo administration of anti-IL-4 MAb increases antilisterial resistance either early or late in infection. In this study, although the number of bacteria in the spleens and livers was decreased on day 2 of infection by in vivo administration of 1 mg of anti-IL-4 MAb, the decrease was not statistically significant, compared with that of the NRG-injected mice (Fig. 3). It is unlikely that the difference between our present results and those of others (10, 36) might be explained by the insufficient injected dose of anti-IL-4 MAb used herein because no significant differences of the bacterial growth in the spleens and livers were observed in mice which were injected with 100 ng to 2 mg of anti-IL-4 MAb (data not shown). Invalidity of anti-IL-4 MAb administration may be attributed to the insufficient production of endogenous IL-4 by *L. monocytogenes* infection because anti-IL-4 MAb clearly affected antilisterial resistance in the anti-IFN-γ MAb-pretreated mice in which the high titers of endogenous IL-4 were detected (Fig. 5).

Neutralization of endogenous IL-10 reportedly enhances host resistance against infections with some pathogens, including *Mycobacterium avium* (2, 4) and *Candida albicans* (31). Moreover, in vitro studies with *L. monocytogenes* showed that IL-10 inhibits both listericidal activity and production of tumor necrosis factor and IL-12 by macrophages and IFN-γ production by natural killer cells (9, 38). In the present study, however, in vivo administration of anti-IL-10 MAb resulted in increased growth of *L. monocytogenes* cells in the organs on

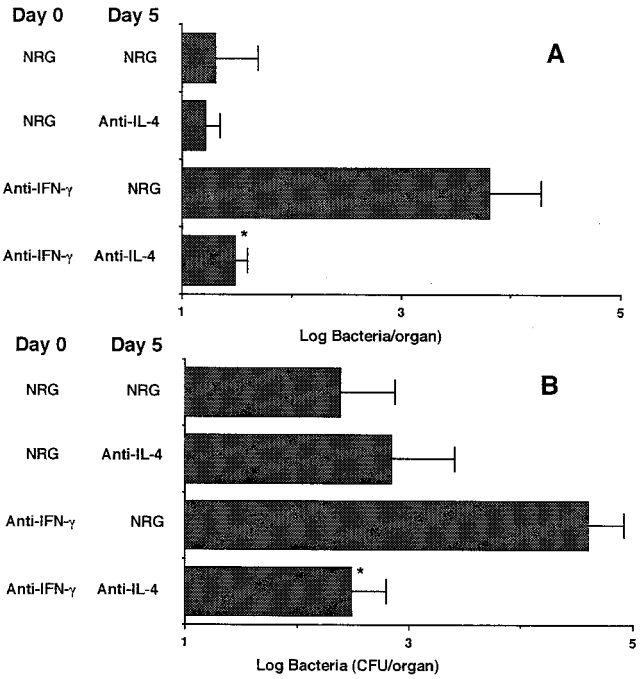

FIG. 8. Effect of in vivo administration of anti-IL-4 MAb on host resistance to *L. monocytogenes* infection in NRG-pretreated or anti-IFN-γ MAb-pretreated mice. Mice were injected with NRG or anti-IFN-γ MAb 2 h before infection. These animals were further injected with NRG or anti-IL-4 MAb 5 days later. The numbers of bacteria in the spleens (A) and livers (B) of four groups were determined on day 8 of infection. Each result represents the mean ± standard deviation for a group of five mice. An asterisk indicates a significant difference from the value for the NRG-injected mice at a P of <0.01.

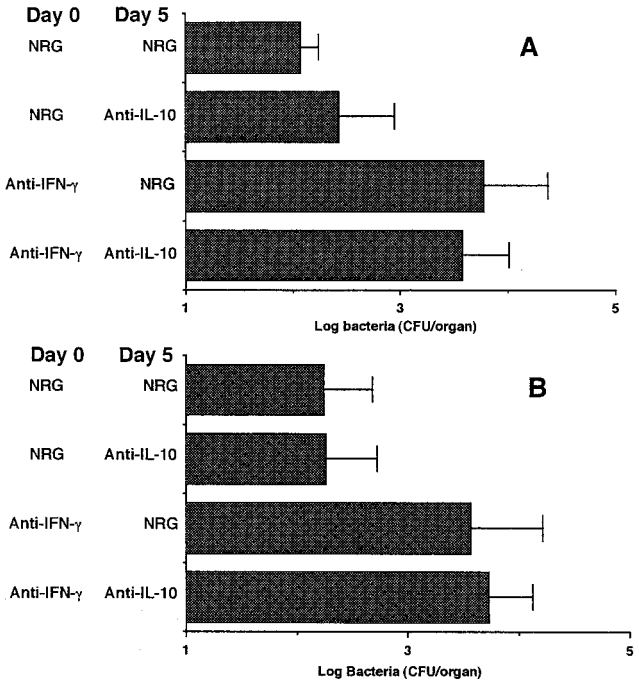

FIG. 9. Effect of in vivo administration of anti-IL-10 MAb on host resistance to *L. monocytogenes* infection in NRG-pretreated or anti-IFN-γ MAb-pretreated mice. Mice were injected with NRG or anti-IFN-γ MAb 2 h before infection. These animals were further injected with NRG or anti-IL-10 MAb 5 days later. The numbers of bacteria in the spleens (A) and livers (B) of four groups were determined on day 8 of infection. Each result represents the mean ± standard deviation for a group of five mice.

day 5 of infection (Fig. 3). Wagner et al. (40) also reported that anti-IL-10 MAb reduced antilisterial resistance late in infection and that liver damage was decreased in the treated mice. They suggested that local IL-10 production might be involved in recruitment of inflammatory cells in granuloma lesions of the liver. We estimated the regulation of endogenous cytokine production by IL-10. However, the titers of tumor necrosis factor, IFN-γ, and IL-6 in the bloodstreams, spleens, and livers of anti-IL-10 MAb-treated mice were comparable to those of NRG-treated mice (data not shown). On the other hand, IL-10 is reportedly a proliferation and differentiation factor for $CD8^+$ cells, increasing both the precursor frequency and the cytolytic activity in vitro (30, 41). Zheng et al. (43) reported that administration of a noncytolytic construct of IL-10 and mouse immunoglobulin Fcγ2a into mice resulted in the enhanced cytolytic activity of allograft-destroying cytotoxic T cells to accelerate rejection of allogeneic islet transplantation. $CD8^+$ cells, which can lyse *L. monocytogenes*-infected target cells, play an important role in antilisterial resistance (17, 21). Therefore, IL-10 may be involved in generation of *L. monocytogenes*-specific cytolytic T cells or enhancement of the cytolytic activity.

In anti-IFN-γ MAb-pretreated mice, the high titers of endogenous IL-4 and IL-10 were induced in the bloodstreams and spleens after day 5 of infection (Fig. 5 and 6), suggesting that the host immune responses were polarized into the Th2 type in IFN-γ-depleted mice. Production of IL-4 and IL-10 by Th0-type and Th2-type $CD4^+$ T cells is well known (22, 35). IL-4 is reportedly produced by $CD4^+NK1.1^+$ T cells (42), and IL-10 is mainly produced by macrophages (9). T-cell receptor γδ T cells (7) and B cells (33) have been also reported to produce IL-4 and IL-10, respectively, in response to *L. monocytogenes* infection. Moreover, Sad et al. (32) showed that $CD8^+$ T cells differentiate into Th2 cytokine-producing cytotoxic cells in the presence of IL-4 and anti-IFN-γ MAb in vitro. Our present study suggested that both $CD4^+$ cells and $CD8^+$ cells might be responsible for the in vivo production of IL-4 and IL-10 in anti-IFN-γ MAb-pretreated mice (Fig. 7). Alternative depletion of $CD4^+$ or $CD8^+$ cells in vivo resulted in a marked reduction of the titers of IL-4 and IL-10, suggesting that these cytokines might not be produced by $CD4^+$ cells and $CD8^+$ cells independently but might be produced by collaboration between these T-cell subsets.

In vivo neutralization of endogenous IL-4, which was induced late in infection, with the specific MAb resulted in cancellation of the suppressed antilisterial resistance in anti-IFN-γ MAb-pretreated mice (Fig. 8). This result suggested that IL-4 plays a crucial role in suppression of antilisterial resistance in anti-IFN-γ MAb-pretreated mice. The suppressive effect of IL-4 is unlikely to relate to the collaborative action of IL-10 (Fig. 9). IL-4 may directly inhibit effector cells such as the deactivation of macrophages (18, 19). In contrast with IL-4, no evidence that IL-10 is involved in suppression was obtained (Fig. 9), in spite of the high production. Hsieh et al. (13) reported that IL-4 is able to inhibit the development of the Th1 phenotype and to drive the development of the Th2 phenotype but that IL-10 is only able to inhibit the development of the Th1 phenotype. The inhibitory effect of IL-10 is dependent on macrophages, and the action is derived from blocking IL-12 production by macrophages (14, 20). Therefore, IL-10 might act as a regulatory molecule early in infection, if the high titer of IL-10 was produced at that stage, whereas IL-10 might not be able to exhibit the inhibitory effect in antilisterial resistance which had been already established, even if excess amounts were produced (8).

Our previous study (27) showed that IFN-γ-deficient mice, in which IFN-γ-producing cells including natural killer cells and $CD4^+$ T cells had been depleted, could exhibit normal antilisterial resistance. Moreover, Harty and Bevan (11) demonstrated that specific antilisterial immunity could be acquired in IFN-γ gene knockout mice. In this study, the restoration of antilisterial resistance by anti-IL-4 MAb treatment in anti-IFN-γ MAb-pretreated mice was not responsible for the induction of endogenous IFN-γ production because IFN-γ was never detected in the bloodstreams and spleens of the MAb-pretreated mice before or after anti-IL-4 MAb administration (data not shown). These results further support the possibility that the generation and expression of acquired antilisterial immunity might be induced even in the absence of IFN-γ.

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