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Treatment with Anti-Interleukin-10 Monoclonal Antibody Enhances Early Resistance to but Impairs Complete Clearance of *Listeria monocytogenes* Infection in Mice

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Mice that received an anti-interleukin-10 (anti-IL-10) neutralizing monoclonal antibody (MAb) (SXC-1) prior to infection with *Listeria monocytogenes* initially demonstrated resistance to the infection, as indicated by reduced recovery of *L. monocytogenes* from their spleens and livers during the first 5 days after challenge. Anti-IL-10 MAb-treated mice then demonstrated reduced resistance during the later stage of infection, as indicated by persistent infection with *L. monocytogenes* in their livers 11 days after challenge. Aspartate aminotransferase (AST) levels (a measure of liver damage) in the sera of control mice increased between 1 and 5 days after challenge, while anti-IL-10 MAb-treated mice maintained lower AST levels. At 7 days after challenge, AST levels in the sera of control mice decreased as the numbers of organisms declined. In contrast, AST levels increased as the infections persisted in anti-IL-10 MAb-treated mice. The AST levels in serum reflected liver histopathology as anti-IL-10 MAb-treated mice exhibited fewer granulomatous lesions and less necrosis of liver tissue than the control mice during the first 5 days after challenge. Anti-IL-10 MAb treatment altered the expression of inflammatory cytokine mRNAs during *L. monocytogenes* infection. Control MAb-treated mice exhibited increased expression of tumor necrosis factor alpha and granulocyte-macrophage colony-stimulating factor mRNA in their livers during *L. monocytogenes* infection, but this increase did not occur in anti-IL-10 MAb-treated mice. Gamma interferon mRNA expression in the livers of the control MAb-treated mice was increased between 1 and 5 days after *L. monocytogenes* challenge and then decreased at 7 days after challenge. In contrast, gamma interferon mRNA expression in the livers of anti-IL-10 MAb-treated mice was not decreased until 7 days after challenge. These results indicate that endogenous IL-10 has both beneficial and detrimental effects on the host response to *L. monocytogenes* infection in mice.

Experimental murine listeriosis is a useful model for studying cytokine regulation of cell-mediated immunity to infection with intracellular pathogens. Certain cytokines (e.g., gamma interferon [IFN-γ] and tumor necrosis factor alpha [TNF-α]) are associated with resistance to *Listeria monocytogenes* infections (22). While these cytokines are involved in activation of the antilisterial host response, other cytokines may block this activation. For example, we recently reported that endogenous interleukin-4 (IL-4) antagonizes host resistance to *L. monocytogenes* infection (15). By neutralizing IL-4 in vivo with a monoclonal antibody (MAb), we observed reduced replication of bacteria in the spleens and livers of *L. monocytogenes*-infected mice. Other cytokines that suppress cell-mediated immunity, one of which is IL-10, have been recognized.

IL-10 is produced by the Th2 subset of helper T cells (11, 27), macrophages (7), and B cells (23). In general, it has been shown to inhibit inflammation and cellular immunity. IL-10 inhibits production of IFN-γ and IL-2 by Th1 helper T cells (13, 19, 28), production of TNF-α by macrophages and other antigen-presenting cells (2, 7, 25, 29), and macrophage cytotoxicity (24, 26). In contrast with these anti-inflammatory properties, IL-10 has been reported to recruit inflammatory cells in vivo. This was best demonstrated in IL-10 transgenic mice, in which pancreatic tissue produced IL-10 and exhibited localized accumulations of lymphocytes and macrophages (31).

We recently reported that IL-10 mRNA-expressing cells were increased in the livers of mice infected with *L. monocytogenes* (30). This finding suggested to us that endogenous IL-10 might dampen the protective host response to listeriosis. In the present study, we investigated the effect of partial neutralization of endogenous IL-10 on the host response to *L. monocytogenes* infection and on regulation of IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF-α mRNA expression. We present evidence that the treatment of mice with anti-IL-10 MAb caused reduced proliferation of *L. monocytogenes* in the spleen and the liver. However, we also made the unexpected observation that anti-IL-10 MAb treatment caused persistence of the infection in the liver. These findings suggest that the role of IL-10 in host defense may be more complex than previously expected.

MATERIALS AND METHODS

Experimental design. Six- to eight-week-old male BDF_1 $(C57BL/6 \times DBA/2)F_1$ mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). The mice were housed under microisolator caps (Lab Products, Frederick, Md.) at the animal care facility of the School of Veterinary Medicine, University of Wisconsin. The mice were allowed to acclimate to the animal care facility for at least 1 week before use in an experiment.

For time course experiments, each mouse was injected

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intraperitoneally (i.p.) with a single dose of 100 μg of anti-IL-10 MAb SXC-1 (21) (a kind gift from the DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, Calif.) or control rat immunoglobulin (rat immunoglobulin G or the immunoglobulin M isotype-matched MAb J5/D, a gift from DNAX). The mice were challenged 2 h later by intravenous (i.v.) injection with 4×10^4 CFU of *L. monocytogenes* EGD. Groups of mice were euthanized by cervical dislocation at 1, 3, 5, 7, 9, and 11 days after *L. monocytogenes* challenge, and approximately one-fourth of each spleen and liver was removed to a sterile glass tissue grinder containing 5 ml of sterile distilled water. The organs were homogenized, serially diluted, and plated on sheep blood agar for bacterial enumeration. Separate samples of livers and spleens were removed for histopathology and RNA extraction. Blood was collected and used to prepare sera that were stored at −20°C for later analysis of IL-10 and aspartate aminotransferase (AST) enzyme activity.

Semiquantitative RT-PCR analysis of cytokine mRNA. Approximately one-fourth of each liver was used for reverse transcription (RT)-PCR analysis of relative changes in the expression of mRNAs of the cytokines IFN-γ, TNF-α, IL-10, and GM-CSF. In our procedure, 0.5 g of each liver sample was homogenized in 0.5 ml of lysis solution (4 M guanidinium isothiocyanate, 0.5% *N*-lauroylsarcosine, 25 mM sodium citrate, 100 mM 2-mercaptoethanol) and extracted with phenol-chloroform solution as described previously (30). The aqueous extracts were precipitated with 0.6 volumes of isopropanol, and the resultant RNA pellets were washed with 70% ethanol. RNA was resuspended in water, quantified by optical density readings at 260 nm, and frozen at −70°C. Integrity of RNA samples was monitored by agarose gel electrophoresis.

For first-strand cDNA synthesis, a 10-μg aliquot of each RNA sample was annealed with 1 μg of oligo$(dT)_{15}$ primer (Promega Corp., Madison, Wis.), mixed with 1 mM deoxynucleoside triphosphates (dNTPs)–35 U of Rnasin (Promega)–525 U of Moloney murine leukemia virus reverse transcriptase in RT buffer (50 mM Tris [pH 8.3], 5 mM KCl, 3 mM $MgCl_2$), and then incubated at 42°C for 2 h. Each cytokine PCR was run with 1 μl of the cDNA products, using cytokine primers obtained from Clonetech Laboratories, Inc. (Palo Alto, Calif.) according to the manufacturer's instructions. Expression of glyceraldehyde-3-phosphate dehydrogenase was assessed from each sample as an internal standard control of sample variation and amplification efficiency. The primers were added to the cDNA template at a 1 μM final concentration along with 200 μM each of the four dNTPs–1.25 U of *Taq* DNA polymerase (Promega) in 50 μl of 10 mM Tris buffer (pH 8.8) (containing 50 mM KCl, 1.5 mM $MgCl_2$, and 1% Triton X-100). Thirty cycles of PCR were run; each cycle consisted of 1-min denaturation at 94°C, 2-min annealing at 60°C, and 3-min extension at 72°C. Two-microliter aliquots of the PCRs were tested for specificity by agarose gel electrophoresis. The remaining PCR products were used for slot blot hybridization analysis.

For slot blot hybridization analysis, the PCR products were denatured in 0.2 N NaOH at 65°C for 30 min and then neutralized with 1 volume of 2 M ammonium acetate. This was applied to a nitrocellulose membrane presoaked in 1 M ammonium acetate with a slot blot apparatus (Schleicher and Schuell, Keene, N.H.). The membrane was baked for 1 h in a vacuum oven at 80°C and prehybridized at 55°C for 1 h in prehybridization buffer (6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 10 mM EDTA [pH 7.5], 2× Denhardt's solution, 100 μg of sheared salmon sperm DNA per ml, and 1% sodium dodecyl sulfate [SDS]). Oligonucleotide probes (10 pg) were radioactively labelled with 5 μCi of [γ-^{32}P]ATP (6,000 Ci/mmol; Du Pont, New England Nuclear, Wilmington, Del.) by using 10 U of T4 polynucleotide kinase (Promega) incubated in the manufacturer's supplied buffer for 30 min at 37°C. After labelling, the probes were separated from unincorporated nucleotide by chromatography in Quick-Spin columns (Boehringer Mannheim, Indianapolis, Ind.). After prehybridization, 4×10^6 cpm of ^{32}P-labelled oligonucleotide probe (Clonetech) per ml was added, and hybridization was allowed to occur overnight at 55°C. Posthybridization washes were done twice at room temperature for 30 min each and once at 55°C for 20 min in 2× SSC with 0.1% SDS. The radioactivity bound in each slot position was measured with radioanalytic imager (Ambis, Inc., San Diego, Calif.), and the data were expressed as counts per minute.

AST assays of liver damage. Blood samples were taken from the mice by cardiac puncture. The serum was separated from the clotted blood after centrifugation at $1,000 \times g$ for 10 min. AST (EC 2.6.1.1) in serum assays were performed according to the manufacturer's instructions with a diagnostic transaminase reagent kit (Sigma Chemical Company, St. Louis, Mo.). Units of transaminase activity were determined by extrapolation from a standard curve generated with known concentrations of sodium pyruvate.

IL-10 ELISA. We designed a sandwich enzyme-linked immunosorbent assay (ELISA) for measurement of IL-10 concentrations in serum. Recombinant murine IL-10, generously provided by DNAX, was used as a standard reference material. To perform this procedure, we coated the wells of Immulon II plates (Dynatech, Alexandria, Va.) with a capture antibody (SXC-1) in 0.1 M sodium bicarbonate buffer (pH 8.4) at a concentration of 10 μg/ml overnight at 4°C. Standards and samples were diluted in phosphate-buffered saline (PBS)–Tween (20 mM Na_2HPO_4, 150 mM NaCl [pH 7.4], 0.05% Tween 20) and incubated in the wells for 2 h at room temperature. The plates were washed three times with PBS-Tween, and then biotinylated anti-IL-10 MAb SXC-2 (5 μg/ml) (Pharmingen, San Diego, Calif.) was added to the wells. The plates were incubated for 1 h at room temperature and then washed three times with PBS-Tween. Ultravidin-alkaline phosphatase (Leinco, St. Louis, Mo.) conjugate was added (1:3,500 dilution), and the plates were incubated for 30 min at room temperature. The plates were washed five times with PBS-Tween, and 100 μl of Attophos substrate (JBL Scientific, San Luis Obispo, Calif.) per well was added according to the procedure of Cano et al. (3). The plates were immediately placed in a fluorescent plate reader (Cytofluor; Millipore Corp., Bedford, Mass.), and fluorescence was measured at an excitation wavelength of 450 nm and an emission wavelength of 580 nm. The limit of sensitivity of this method was approximately 0.065 U of recombinant IL-10.

Statistical analyses. Data were analyzed by a one-way analysis of variance followed by the Tukey modification of paired *t* tests as performed by the Instat statistical software package (GraphPad, San Diego, Calif.).

RESULTS

Treatment with anti-IL-10 MAb enhances early resistance to *L. monocytogenes* infection. Mice were treated with the anti-IL-10 neutralizing MAb SXC-1 or the isotype-specific control MAb J5/D, 2 h prior to challenge with *L. monocytogenes*, and then euthanized at 1, 3, 5, and 7 days after challenge. The effect of anti-IL-10 MAb treatment was apparent earlier in spleens than in livers. A separate experiment was conducted to assess the early effect of anti-IL-10 MAb treatment on the host

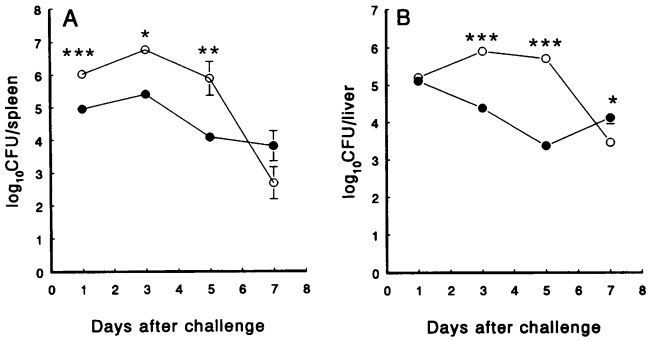

FIG. 1. Administration of anti-IL-10 MAb enhances the early resistance of mice to *L. monocytogenes* infection. Anti-IL-10 MAb-treated (●) and control antibody-treated (○) mice received 0.1 mg of MAb 2 h before challenge with 4×10^4 CFU of *L. monocytogenes*. Portions of the spleen (A) and liver (B) from each mouse were removed and homogenized separately in 5 ml of sterile water with sterile tissue grinders. A 0.1-ml aliquot of each homogenate was serially diluted and plated onto Trypticase soy agar supplemented with 5% sheep blood. Data are the mean ± SEM $\log_{10}$ CFU of *L. monocytogenes* recovered from each treatment group (four mice per group). Where error bars are not shown, the values were smaller than the width of the symbols (range, 0.02 to 0.13 $\log_{10}$). Anti-IL-10 MAb-treated mice were statistically different than control mice at the time points with asterisks. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.)

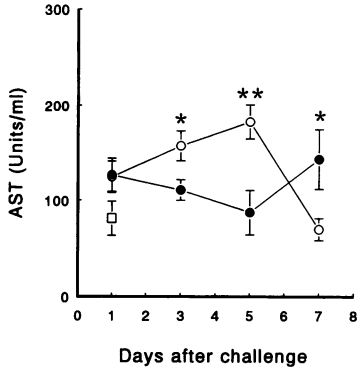

FIG. 2. AST levels in the serum of anti-IL-10 MAb-treated and control antibody-treated *L. monocytogenes*-infected mice. Groups of mice were injected i.p. with 0.1 mg of anti-IL-10 MAb (●) or control antibody (○) and then challenged i.v. with 4×10^4 CFU of *L. monocytogenes*. Mice were euthanized at 1, 3, 5, and 7 days after challenge. Blood was collected from each mouse by cardiac puncture and used to prepare sera that were analyzed for AST levels. The range of AST values for serum from 10 normal BDF_1 mice are indicated (□). The single asterisks indicate $P < 0.05$ and the double asterisk indicates $P < 0.01$ for the treatment group versus the control at that time point.

response to *L. monocytogenes* infection. At 4 h after challenge, anti-IL-10 MAb-treated mice and control antibody-treated mice had equivalent numbers of *L. monocytogenes* organisms in their spleens and livers (3.18 ± 0.09 $\log_{10}$ CFU per spleen and 4.15 ± 0.08 $\log_{10}$ CFU per liver for anti-IL-10 MAb-treated mice versus 3.17 ± 0.05 $\log_{10}$ CFU per spleen and 4.24 ± 0.11 $\log_{10}$ CFU per liver for control mice). During the 7-day time course, the spleens of anti-IL-10 MAb-treated mice had 1 $\log_{10}$ fewer *L. monocytogenes* organisms at 1 and 3 days after challenge than those of control mice (Fig. 1A). At 3 days after challenge, the livers of anti-IL-10 MAb-treated mice exhibited a 2 $\log_{10}$ reduction compared with those of controls (Fig. 1B), and their spleens yielded 2 $\log_{10}$ fewer *L. monocytogenes* organisms than those of control mice. At 5 days after challenge, anti-IL-10 MAb-treated mice continued to have significantly fewer *L. monocytogenes* organisms in their spleens and livers than control mice did. After day 5, however, the relationship between the two groups of mice reversed itself. The numbers of *L. monocytogenes* organisms in anti-IL-10 MAb-treated mice increased, whereas the numbers of *L. monocytogenes* organisms in the spleens and livers of control mice decreased. The observed effects of anti-IL-10 MAb treatment were at least partially due to neutralization of endogenous IL-10, as determined by measurement of IL-10 levels in serum by ELISA at 1 day after challenge. A fourfold reduction of the IL-10 levels in the serum of anti-IL-10 MAb-treated mice compared with those of controls was observed (1.0 ± 0.9 and 4.3 ± 2.2 IU/ml, respectively). IL-10 levels in serum were not decreased by treatment with the J5/D control antibody. The effects of anti-IL-10 MAb treatment on IL-10 levels in serum were transient, being comparable with the controls by 3 days after challenge.

Treatment of mice with anti-IL-10 MAb before *L. monocytogenes* challenge causes reduced liver damage. Liver damage in *L. monocytogenes*-infected mice was assessed in two ways, measurement of AST levels in serum and histopathology. We determined the average range (mean ± standard error of the mean [SEM]) of AST levels in the serum of 10 normal BDF_1 mice to be 91 ± 18 U/ml. Mice treated with anti-IL-10 MAb had significantly lower AST levels in their serum during the first 5 days after *L. monocytogenes* challenge than control mice (Fig. 2). By 7 days after challenge, AST levels in the serum of control mice were decreasing, while they were increasing in that of anti-IL-10 MAb-treated mice. The late onset of liver damage in anti-IL-10 MAb-treated mice reflected the persistence of *L. monocytogenes* infection in these mice.

Histopathological differences in the livers of anti-IL-10 MAb-treated and control mice were observed during the course of the infection. One day after challenge, the livers of both groups of mice contained small granulomatous lesions with similar infiltration of inflammatory cells in the sinuses (Fig. 3A and B). Three days after challenge, extensive hepatic sinus infiltration by macrophages and neutrophils and large granulomatous lesions were present in the livers of control *L. monocytogenes*-infected mice (Fig. 3C). In contrast, the livers of anti-IL-10 MAb-treated mice had diffuse inflammatory cell infiltration of the sinuses, which consisted mostly of macrophages, and had fewer, smaller granulomatous lesions (Fig. 3D). There were noticeably fewer neutrophils in the livers of anti-IL-10 MAb-treated mice than in those of control mice. Similar differences in liver pathology were seen at 5 days after challenge (Fig. 3E and F). On day 7, the control mice had fewer granulomatous lesions and less inflammatory cell infiltration in the sinuses than at earlier time points (Fig. 3G). In addition, numerous mitotic figures were present in the hepatocytes, indicating tissue regeneration and repair. The livers of anti-IL-10 MAb-treated *L. monocytogenes*-infected mice still exhibited a few small granulomatous lesions and diffuse inflammatory cell infiltration of the sinuses (Fig. 3H). Occasional necrotic hepatocytes were observed, suggesting that liver damage was continuing.

Anti-IL-10 MAb treatment before challenge with *L. monocytogenes* altered expression of inflammatory cytokine mRNA. We have previously reported that mRNA expression of the inflammatory cytokines TNF-α, GM-CSF, and IFN-γ in the liver is increased after challenge with *L. monocytogenes* (30). In the present study, we observed increased expression of TNF-α and GM-CSF mRNA in control mice at 5 and 7 days after *L.*

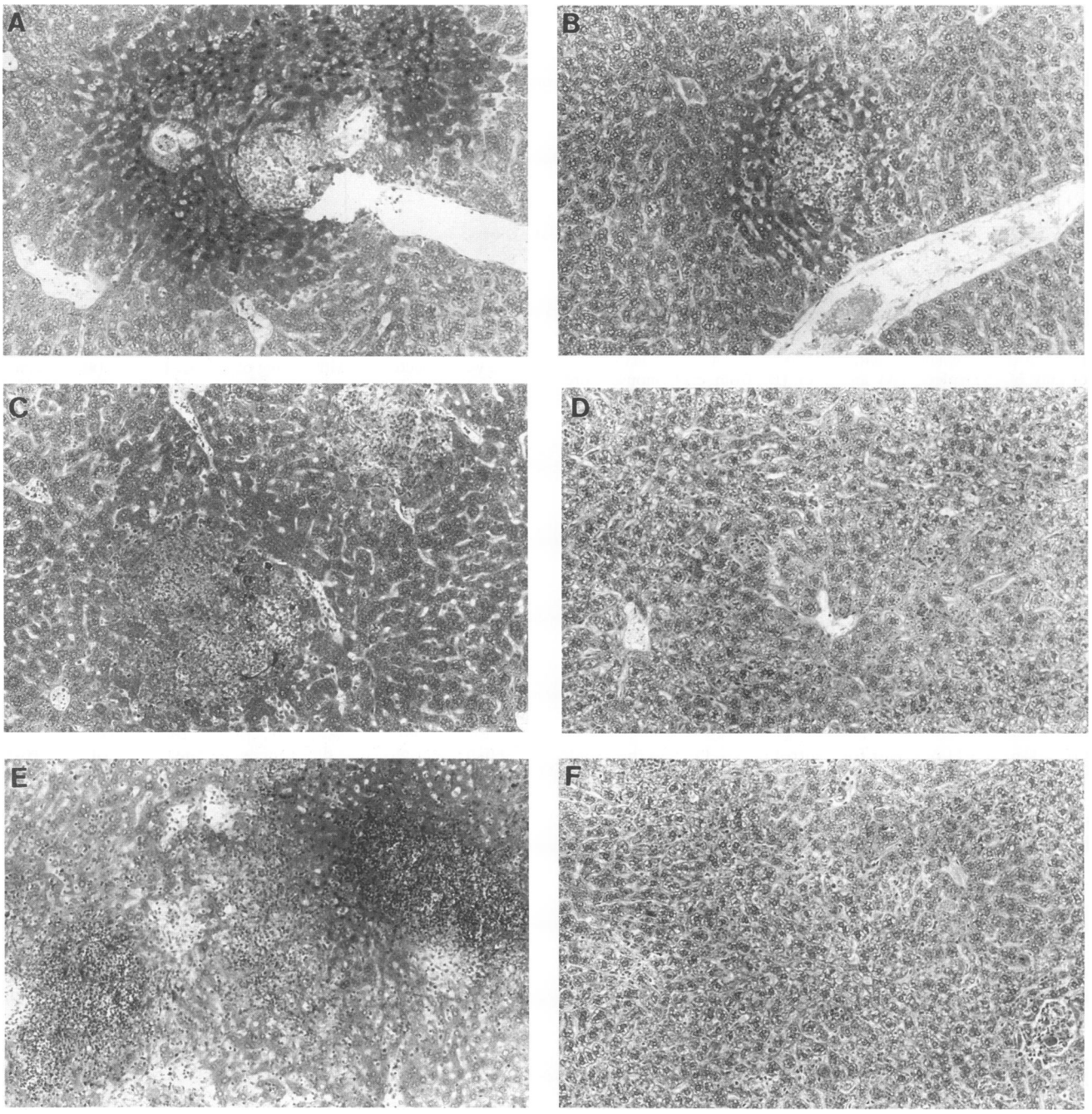

FIG. 3. Differences in liver pathology were observed between control and anti-IL-10 MAb-treated mice during the course of *L. monocytogenes* infection. Groups of mice were injected i.p. with anti-IL-10 MAb (0.1 mg) or control antibody and then challenged i.v. with 4×10^4 CFU of *L. monocytogenes*. Mice were euthanized at 1, 3, 5, and 7 days after challenge, and portions of their livers were fixed, sectioned, and stained with hematoxylin and eosin. One day after challenge, control (A) and anti-IL-10 MAb-treated (B) mice had similar amounts of inflammatory cell infiltration and similar numbers of pyogranulomatous lesions. At 3 days after challenge, control livers (C) had more large granulomatous lesions than those of anti-IL-10 MAb-treated mice (D), which exhibited more diffuse inflammatory cell infiltration. Similar differences in liver damage between control (E) and anti-IL-10 MAb-treated (F) mice were observed at 5 days after challenge. At 7 days after challenge, the control mice (G) had fewer large granulomatous lesions than at day 5. In addition, there were numerous hepatocytes with mitotic figures (arrows), indicating tissue repair. In contrast, the anti-IL-10 MAb-treated mice (H) continued to exhibit small granulomatous lesions with a diffuse cell infiltrate consisting predominantly of macrophages. Original magnification, ×125.

monocytogenes infection (Fig. 4A and B). In contrast, expression of TNF-α and GM-CSF mRNA was not appreciably elevated in anti-IL-10 MAb-treated mice. Control *L. monocytogenes*-infected mice expressed greatly increased IFN-γ mRNA levels at 3 days after challenge, which gradually decreased thereafter. In contrast, anti-IL-10 MAb-treated mice exhibited a slower increase in expression of IFN-γ mRNA that continued through 7 days after challenge (Fig. 4C).

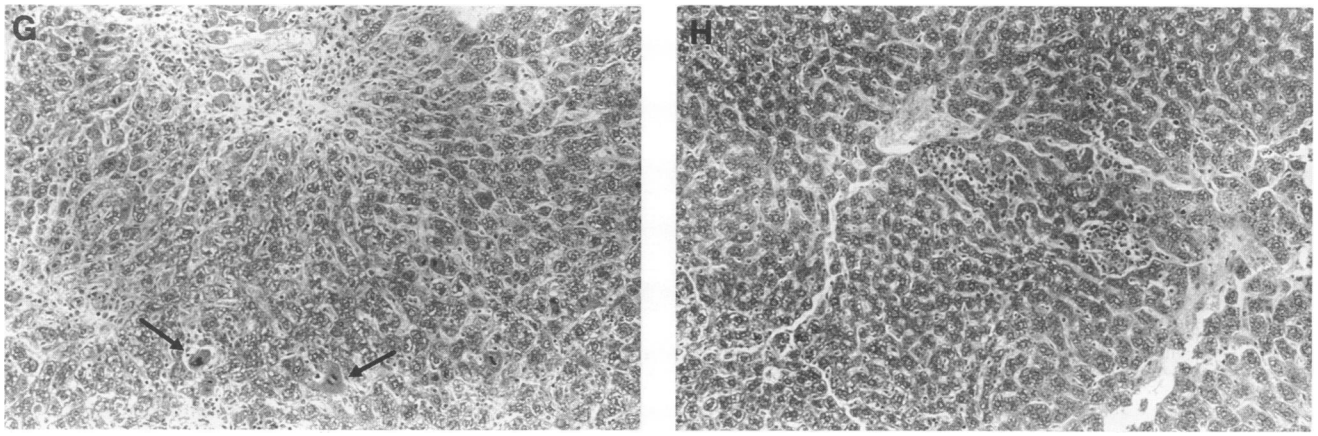

FIG. 3—*Continued.*

Treatment of mice with anti-IL-10 MAb did not affect the level of IL-10 mRNA expression in the liver during *L. monocytogenes* infection (Fig. 4D). Previous in vitro studies suggested autocrine suppression of IL-10 mRNA transcription by IL-10 (7). Thus, our observation could indicate that the level of IL-10 neutralization achieved in vivo in the present study was insufficient to increase IL-10 transcription or that there is some other mediator that has a negative regulatory effect on IL-10 mRNA transcription.

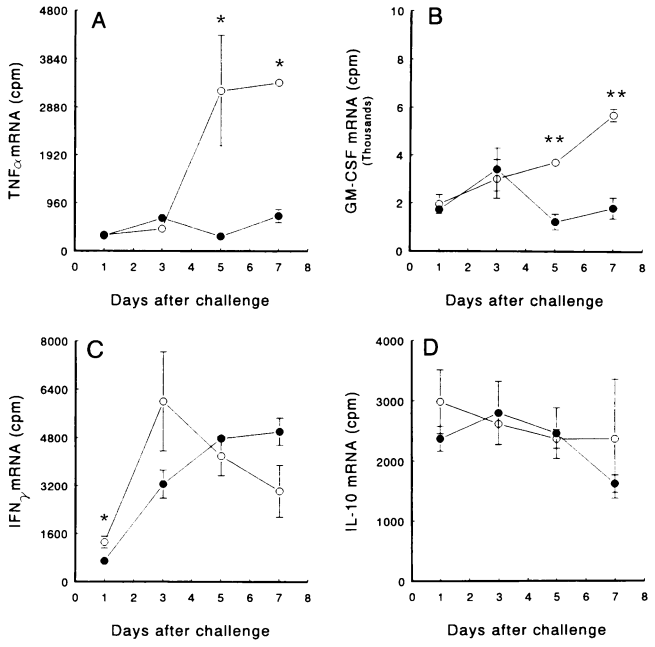

FIG. 4. Cytokine mRNA expression during the course of *L. monocytogenes* infection. Total RNA samples were prepared from the livers of anti-IL-10 MAb-treated (●) and control MAb-treated (○) mice. RT-PCR analysis was performed on the samples with specific primers for TNF-α (A), GM-CSF (B), IFN-γ (C), and IL-10 (D). The PCR products were blotted onto nitrocellulose filters and hybridized with ^{32}P-labelled oligonucleotide probes internal to the amplified sequences as described in Materials and Methods. Data are the mean ± SEM counts per minute of bound radioactivity (four mice per group). Where error bars are not visible, they were smaller than the width of the symbols. Anti-IL-10 MAb-treated mice were statistically different from control mice at the time points with asterisks. *, $P < 0.05$; **, $P < 0.001$.

We also measured the relative differences in expression of IL-2, IL-5, IL-6, and transforming growth factor β (TGF-β) mRNA expression during *L. monocytogenes* infection in control and anti-IL-10 MAb-treated mice. As indicated in Table 1, anti-IL-10 MAb treatment resulted in significantly decreased expression of IL-2 mRNA, compared with that of control mice at 1 day after challenge. Both groups of mice expressed similar levels of IL-2 mRNA at 3, 5, and 7 days after challenge. Expression of IL-5 mRNA did not change significantly during the course of *L. monocytogenes* infection in either treatment group. Expression of IL-6 mRNA did not change in anti-IL-10 MAb-treated mice, but it increased significantly at 5 days after challenge in control mice. Expression of TGF-β mRNA increased in control mice at 5 days after challenge and subsequently decreased at 7 days after challenge. In contrast, TGF-β mRNA expression remained quite low in the livers of anti-IL-10 MAb-treated mice between 1 and 5 days after challenge. At 7 days after challenge, TGF-β mRNA expression was markedly increased in anti-IL-10 MAb-treated mice, similar to the pattern of IFN-γ mRNA expression observed in these mice.

Treatment with anti-IL-10 MAb before *L. monocytogenes* infection leads to persistent infection. As described earlier,

TABLE 1. Expression of IL-2, IL-5, IL-6, and TGF-β mRNA in control and anti-IL-10 MAb-treated mice during *L. monocytogenes* infection

Cytokine mRNA	Murine group	Hybridized probe (cpm, 10^2) at day after challenge[a]:			
		1	3	5	7
IL-2	Control	55.2 ± 11.3[b]	27.0 ± 3.6	32.2 ± 2.6	19.6 ± 4.6[b]
	Anti-IL-10	28.2 ± 2.3	25.9 ± 3.7	27.8 ± 7.7	10.3 ± 1.8
IL-5	Control	8.4 ± 3.4	4.7 ± 0.6	4.0 ± 0.5	3.8 ± 2.9
	Anti-IL-10	4.7 ± 1.1	4.8 ± 1.6	4.6 ± 1.2	1.5 ± 0.4
IL-6	Control	2.3 ± 0.2	2.5 ± 0.4	4.7 ± 1.4[b]	2.2 ± 0
	Anti-IL-10	2.1 ± 0.1	3.4 ± 0.4	2.1 ± 0.3	3.4 ± 0.1
TGF-β	Control	1.5 ± 0.1	0.7 ± 0.3	6.4 ± 0.9[b]	2.6 ± 1.4[b]
	Anti-IL-10	1.0 ± 0.3	0.4 ± 0.1	0.7 ± 0.2	6.7 ± 2.1

[a] Data are means ± SEM of samples from four mice.
[b] $P < 0.05$.

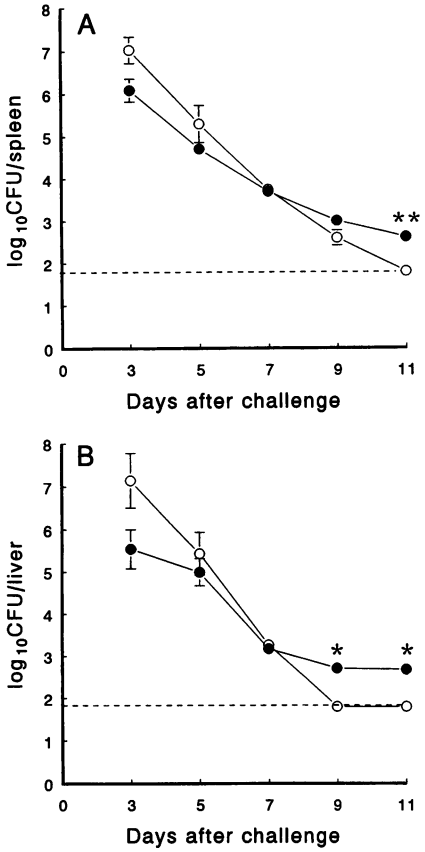

FIG. 5. Administration of anti-IL-10 MAb resulted in persistence of *L. monocytogenes* in the spleens and livers of mice through 11 days after challenge. Groups of four mice were injected i.p. with 0.1 mg of anti-IL-10 (●) or control antibody (○) and then challenged i.v. with 4×10^4 CFU of *L. monocytogenes*. Mice were euthanized at 3, 5, 7, 9, and 11 days after challenge, and portions of the spleen (A) and liver (B) from each mouse were homogenized, diluted, and plated on Trypticase soy agar with 5% sheep blood to determine the CFU of *L. monocytogenes*. Data are the mean ± SEM $\log_{10}$ CFU of *L. monocytogenes* recovered. The dashed line indicates the limit of detection. The results for anti-IL-10 MAb-treated mice differed from control mice at the time points with asterisks. *, $P < 0.001$; **, $P < 0.0001$.

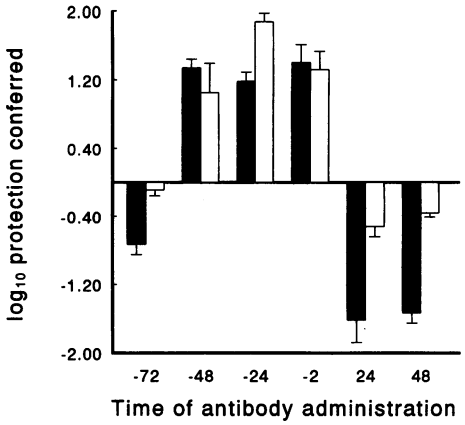

FIG. 6. Administration of anti-IL-10 MAb after *L. monocytogenes* challenge impaired resistance to infection. Groups of mice were challenged i.v. with 4×10^4 CFU of *L. monocytogenes*. Mice received anti-IL-10 Mab or control antibody at 2, 24, 48, or 72 h before and 24 or 48 h after bacterial challenge. The mice were euthanized 3 days after challenge, and their spleens (open bars) and livers (solid bars) were removed, homogenized, diluted, and plated on blood agar to estimate the CFU of *L. monocytogenes* as described in Materials and Methods. Data are the mean ± SEM $\log_{10}$ difference between anti-IL-10 MAb-treated and control mice (four mice per group).

when we treated mice with anti-IL-10 MAb, we observed an early reduction in the numbers of *L. monocytogenes* organisms in the spleen and the liver. In these first experiments, we did not follow the fate of the infection at later time points. To determine the eventual fate of the infection in anti-IL-10 MAb-treated mice, we conducted another time course experiment at 3 to 11 days after challenge. *L. monocytogenes* was completely cleared from the spleens of control mice but not anti-IL-10 MAb-treated mice by 11 days after challenge (Fig. 5A). Similarly, control mice had completely eliminated *L. monocytogenes* from their livers by 9 days after challenge (Fig. 5B), whereas anti-IL-10 MAb-treated mice continued to exhibit infection in their livers at 9 and 11 days after challenge. These results suggest that early neutralization of endogenous IL-10 somehow impairs host defense mechanisms required for complete elimination of *L. monocytogenes* in experimentally infected mice.

Administration of anti-IL-10 MAb after challenge with *L. monocytogenes* caused enhanced bacterial proliferation. Since anti-IL-10 MAb treatment prior to *L. monocytogenes* challenge resulted in persistent infection, we hypothesized that treatment with anti-IL-10 MAb after *L. monocytogenes* challenge would exacerbate the infection. To test this hypothesis, we treated mice with anti-IL-10 MAb at different times before and after i.v. challenge with 4×10^4 CFU of *L. monocytogenes*. As illustrated in Fig. 6, bacterial replication in the liver was enhanced in mice treated with anti-IL-10 MAb at 24 or 48 h after challenge. This is in stark contrast with the reduced bacterial proliferation observed in the livers of mice treated with anti-IL-10 MAb at 2, 24, or 48 h prior to bacterial challenge. An additional experiment was conducted to verify the importance of endogenous IL-10 later in the host response to listeriosis. Mice were challenged with *L. monocytogenes* and treated with anti-IL-10 MAb or control antibody at 1, 3, and 5 days after challenge. The experimental design called for the mice to be euthanized 2 days after MAb treatment. However, the mice treated with anti-IL-10 MAb at 3 and 5 days after challenge died before their scheduled euthanasia at 5 and 7 days, respectively. Control antibody-treated mice exhibited the typical course of infection with predicted numbers of *L. monocytogenes* organisms in their spleens and livers (Table 2). The increased numbers of *L. monocytogenes* organisms in the livers of mice treated with anti-IL-10 MAb after bacterial challenge is consistent with our histopathological evidence that anti-IL-10 MAb treatment impaired the recruitment of inflammatory cells required to eliminate *L. monocytogenes* during the late stage of the infection.

DISCUSSION

We recently observed that IL-10 mRNA-expressing cells increased in number within the livers of mice during *L. monocytogenes* infection (30). This finding compelled us to consider that IL-10 may play a significant role in the host response during listeriosis. On the basis of considerable evidence that IL-10 inhibits functions related to inflammation and cellular immunity, we predicted that endogenous IL-10 would have a negative effect on antilisterial resistance.

TABLE 2. Lethality of treatment of mice with anti-IL-10 MAb during infection[a]

Day of treatment	Day of sampling	MAb	$\log_{10}$ CFU[b]	
			Spleen	Liver
1	3	+	7.49 ± 0.05	7.58 ± 0.30
		−	7.22 ± 0.05	7.15 ± 0.15
3	5	+	†[c]	†
		−	6.46 ± 0.47	7.49 ± 0.65
5	7	+	†	†
		−	3.35 ± 1.03	4.31 ± 0.83

[a] Mice were challenged with 4×10^4 CFU of *L. monocytogenes* by i.v. injection. The anti-IL-10 MAb (+) or control MAb (−) was i.p. injected at the times indicated, and the mice were euthanized at the indicated times. Spleens and livers were cultured as described in Materials and Methods.

[b] Data are the mean ± SEM $\log_{10}$ CFU per organ (four mice per group).

[c] The mice in this group died with $>10^8$ CFU of *L. monocytogenes* per organ before the scheduled sampling date.

In the present study, we treated mice with a neutralizing anti-IL-10 MAb (SXC-1) to investigate the effects of endogenous IL-10 on the host response to *L. monocytogenes* infection. At the time this study was begun, SXC-1 was the only anti-mouse IL-10 MAb described as neutralizing IL-10 in vivo (17, 18). Our results obtained with an IL-10 ELISA suggested that SXC-1 MAb treatment reduced IL-10 levels in serum by nearly 75%. It is unclear whether ELISA levels in serum indicate the availability of biologically active IL-10. It is possible that the ELISA overestimated biologically active IL-10 levels by identifying IL-10 complexed with anti-IL-10 MAb. The best indication of neutralization of IL-10 in the present study was the expected decrease in bacterial numbers in the spleens and livers of anti-IL-10 MAb-treated mice during the early stage of *L. monocytogenes* infection. This observation is consistent with the predicted deleterious effect of Th2 cytokines on cellular immunity (13). In addition, the increased class II major histocompatibility complex (MHC) antigen expression by anti-IL-10 MAb-treated mice was consistent with prior evidence that IL-10 suppresses class II MHC antigen expression in vitro (8).

Our initial observation in the present study was that anti-IL-10 MAb treatment before challenge with *L. monocytogenes* decreased the numbers of *L. monocytogenes* organisms recovered from spleens and livers during the first 5 days of infection. Similar observations have been made recently with *Mycobacterium avium*-infected mice (1, 6). In these two studies, anti-IL-10 antibody treatment at the time of infection reduced the burden of *M. avium* in the spleens and livers of mice by 2 $\log_{10}$ CFU. These authors suggested that induction of IL-10 may be part of the pathogen's strategy to suppress the host resistance to *M. avium*, resulting in prolonged infection (1). Both studies with *M. avium*, and our present study with *L. monocytogenes*, support the hypothesis that endogenous IL-10 can suppress the host response to infection by intracellular pathogens.

One possible way that IL-10 can suppress the host response is by inhibiting the production of IFN-γ, which is important for macrophage activation. There is considerable evidence that IL-10 can suppress Th1-cell production of IFN-γ both directly (11) and by suppression of T-cell activation by antigen-presenting cells (10, 13, 20). However, the results of the present study are not entirely consistent with this function of IL-10. IFN-γ mRNA expression at day 1 was actually higher in the livers of control mice, which had unperturbed levels of IL-10, than in those of anti-IL-10 MAb-treated mice. This argues against a substantial suppression of IFN-γ expression by IL-10. The pattern of IFN-γ expression followed the increase in bacterial burden, suggesting that this produced a stimulus that was stronger than IL-10-mediated suppression.

Most experimental evidence suggests that the suppressive effects of IL-10 on the host response are predominantly mediated by its effects on macrophages. For example, macrophage activation is directly inhibited by IL-10 (2). IL-10 alone, or in synergy with IL-4 and TGF-β, inhibits the cytotoxic activity of macrophages against schistosomula of *Schistosoma mansoni* (24). In a recent study with *L. monocytogenes*-infected mice, bacterial proliferation in the liver was suppressed when mice received exogenous IFN-γ that increased MHC class II antigen expression on antigen-presenting cells that were induced with exogenous IFN-γ (19). IL-10 has been shown to suppress the expression of MHC class II antigens on human monocytes, making them less capable of activation and functioning as antigen-presenting cells (8). In the present study, we noticed that anti-IL-10 MAb-treated mice had significantly increased MHC class II antigen expression (37.3 ± 1.5% $I\text{-}A^{d+}$ macrophages in the spleens of anti-IL-10 MAb-treated mice versus 29.5 ± 2.3% $I\text{-}A^{d+}$ macrophages in the spleens of control antibody-treated mice) on the third day of *L. monocytogenes* infection. Thus, our results suggest that endogenous IL-10, when present in the tissues during the early stages of *L. monocytogenes* infection, modulates some aspects of macrophage activation.

Another effect of IL-10 is inhibition of macrophage cytokine production (25). IFN-γ-activated human monocytes produce IL-1, IL-6, IL-8, TNF-α, GM-CSF, and granulocyte colony-stimulating factor; production of these cytokines was inhibited at the level of transcription by the addition of IL-10 (7). IL-10 has been reported to inhibit TNF-α production by murine and human macrophages (2, 12, 25). In the present study, we observed differential effects on the transcription of TNF-α and GM-CSF in anti-IL-10 MAb-treated *L. monocytogenes*-infected mice compared with that in infected control mice. *L. monocytogenes*-infected control mice exhibited increased expression of TNF-α and GM-CSF at 3 days after challenge, which decreased thereafter. In contrast, TNF-α and GM-CSF mRNA expression were never increased in anti-IL-10 MAb-treated mice. Expression of TNF-α in *L. monocytogenes*-infected mice has been reported to be essential for host resistance (16, 22). In the present study, the coincidental inability to eliminate the infection and failure to induce TNF-α mRNA expression exhibited by anti-IL-10 MAb-treated mice is intriguing. It suggests that endogenous IL-10 activity assisted the induction of TNF-α expression in *L. monocytogenes*-infected mice and that this induction was important to prevent persistent infection. The scope of this study was not broad enough to determine the mechanism by which this occurred.

Perhaps the most significant observation made in this study was the persistence of *L. monocytogenes* in anti-IL-10 MAb-treated mice. We recovered approximately 3 $\log_{10}$ *L. monocytogenes* organisms from the spleens and livers of anti-IL-10 MAb-treated mice at 11 days after challenge, whereas control mice had completely cleared the infection by this time. This inhibition of bacterial clearance may be the result of reduced recruitment of inflammatory cells into granulomatous lesions in the livers of anti-IL-10 MAb-treated mice. This suggests that IL-10 might be involved in the recruitment of the inflammatory cells that form granulomatous lesions in the liver and are an important aspect of the host response in murine listeriosis (14). This hypothesis is consistent with a recent report of neutrophil and macrophage recruitment into a tissue where IL-10 was being produced. In a recent study, Wogensen et al. (31) observed that transgenic mice, which expressed IL-10 in the

pancreas, exhibited scattered infiltration of macrophages and focal accumulations of inflammatory cells. This finding, which suggests that local production of IL-10 can cause inflammatory cell recruitment into a nonlymphoid tissue, is consistent with our observation that recruitment of inflammatory cells (particularly neutrophils) into granulomatous lesions of the liver was impaired by anti-IL-10 MAb treatment of *L. monocytogenes*-infected mice. Perhaps anti-IL-10 MAb treatment inhibited recruitment of neutrophils and other cells into the infected liver where they would destroy infected hepatocytes, as suggested by others (4, 5), or release mediators that prevent intracellular multiplication of *L. monocytogenes*. This hypothesis is consistent with our observation that anti-IL-10 MAb treatment, given 1 or 2 days after challenge, was associated with increased *L. monocytogenes* proliferation in the liver.

In summary, this study provides evidence that endogenous IL-10 has both beneficial and detrimental roles in the host response to *L. monocytogenes* infection. The beneficial function appears to be associated with recruitment of inflammatory cells to foci of infection in the liver. The detrimental role is that endogenous IL-10 appears to suppress resistance during the early stage of infection. IL-10 is a promising anti-inflammatory agent that may be useful therapeutically to suppress transplant rejection and chronic inflammation during autoimmune diseases (9). The potential uses of IL-10 as an anti-inflammatory agent must take into consideration the knowledge that IL-10 may have unexpected biological effects beyond those predicted by its role as an inhibitor of cytokine production by Th1 cells, NK cells, and macrophages.

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